# UNIVERSIDAD COMPLUTENSE DE MADRID

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TESIS DOCTORAL

# Efectos postnatales de la variación de peso al nacimiento en el cerdo ibérico

MEMORIA PARA OPTAR AL GRADO DE DOCTORA
PRESENTADA POR

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MARTA VÁZQUEZ GÓMEZ

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Tesis Doctoral presentada por
MARTA VÁZQUEZ GÓMEZ

Realizada bajo la dirección de los doctores
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MADRID, 2018

La Dra. Beatriz Isabel Redondo, profesora contratada doctor del Departamento de Producción Animal de la Facultad de Veterinaria de la Universidad Complutense de Madrid, el doctor Antonio González de Bulnes, investigador del Departamento de Reproducción Animal del Instituto Nacional de Investigación y Tecnología Agraria y Alimentaria, y la doctora Cristina Óvilo Martín, investigadora del Departamento de Mejora Genética Animal del Instituto Nacional de Investigación y Tecnología Agraria y Alimentaria,

CERTIFICAN:

Que la Tesis Doctoral titulada "EFECTOS POSTNATALES DE LA VARIACIÓN DE PESO AL NACIMIENTO EN EL CERDO IBÉRICO" presentada por Marta Vázquez Gómez para optar al grado de Doctor, ha sido realizada bajo su dirección, cumple las condiciones exigidas para obtener dicho título y autorizan su presentación para que sea juzgada por la comisión correspondiente.

Y para que así conste, firman en Madrid, a 19 de septiembre de 2018.

| | | |
|---|---|---|
| Beatriz<br>Isabel Redondo | Antonio<br>González de Bulnes | Cristina<br>Óvilo Martín |

El trabajo experimental que ha dado lugar a esta memoria ha sido realizado en el Departamento de Producción Animal de la Facultad de Veterinaria de la Universidad Complutense de Madrid y en los Departamentos de Mejora Genérica Animal y Reproducción Animal del Instituto Nacional de Investigación Agraria y Alimentaria, financiado mediante los siguientes proyectos de investigación:

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A mi madre

*Hazlo*
*porque aunque tuvieses toda la libertad y dinero del mundo,*
*seguirías haciendo lo mismo.*
*Trabajar en Ciencia, una Pasión.*

# Receta para una buena tesis

Como todo buen plato culinario una buena tesis requiere de trabajo y atención, pero también de unos ingredientes básicos:

Para empezar, es necesario que alguien deposite en ti su confianza para elaborarla y te guie cuando te pierdas intentando hacerla o yerres. Te mostrarán lo que puedes llegar a hacer, aunque algunas cosas serán tus mayores retos. Para superarlos lo mejor siempre será una buena mezcla de disciplinas, la sinergia siempre enriquece y ayuda a tener perspectiva.

El ambiente de elaboración es muy importante, las personas que lo formen marcarán la diferencia. Con ellas aprenderás los pasos más básicos y cometerás los errores más tontos. También, te ayudarán a resolver desde las preguntas más chorras a las más complicadas. Lo bueno, es que siempre encontrarás una sonrisa y ayuda para solucionarlas. Pero sobretodo, en ellos encontrarás ánimos y apoyo marinado con risas diarias.

En este ambiente también estarán aquellas personas con las que no trabajas codo con codo, pero que en ocasiones (muchas a veces) te ofrecen su tiempo o sus conocimientos para ayudarte en tu camino presente o futuro. Hay que valorar esos detalles.

Descansar para comer siempre será importante, pero es mucho mejor cuando lo haces con gente que está "tesando". Encontrarás respuestas a preguntas que tenías y, también, a las que no sabías que tenías. Reírse de las batallitas contra la burocracia y otras torturas siempre será necesario.

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Como la vida no es sólo trabajo, a veces deberás reposar y dejar las cosas reposar. Para ello nada mejor que esas personas que llevan contigo un montón de tiempo (o no tanto). Ellos te sonreirán y te animarán mientras tú les das la brasa con ese extraño trabajo tuyo, sólo porque es importante para ti.
Los amigos, un condimento imprescindible de la vida.

Por último, todos necesitamos ese soporte diario que hará todo por nosotros. Esas personas claves en la vida que hacen posible que estés a tope con tu pasión. ¡Cuídalas!

Siempre se quedan cosas en el tintero, pero...

¡muchas gracias a todos por estar a mi lado!

^^

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# Abreviaturas

AECERIBER. Asociación Española de Criadores de Ganado Porcino Selecto ibérico Puro y Tronco ibérico

AG. Ácido Graso

APN. Alto Peso al Nacimiento

ASICI. Asociación Interprofesional del Cerdo ibérico

BPN. Bajo Peso al Nacimiento

CE. Comisión Europea

CV. Coeficiente de variación

DE. Desviación estándar

FLN. Fracción de los Lípidos Neutros

FLP. Fracción de los Lípidos Polares

GMDP. Ganancia Media Diaria de Peso

HT. Hidroxitirosol

IC. Índice de Conversión

LD. *Longissimus dorsi*

MAPAMA. Ministerio de Agricultura y Pesca, Alimentación y Medio Ambiente

MBPN. Muy bajo peso al nacimiento

PNM. Peso al Nacimiento Medio

PNN. Peso Normal al Nacimiento

PV. Peso Vivo

PN. Peso al Nacimiento

RCIU. Retraso de Crecimiento Intrauterino

UE. Unión Europea

# Resumen

La optimización de la rentabilidad en la producción porcina se basa principalmente en el aumento de los kilos de carne producidos por cerda y año y en la obtención de la mayor homogeneidad posible en los lotes de crecimiento y sacrificio. Para ello, en razas comerciales genéticamente seleccionadas, se ha buscado el aumento del número de lechones por camada. Sin embargo, las camadas con un alto número de lechones se ven penalizadas por aumentos en la variabilidad de peso al nacimiento y por un mayor porcentaje de lechones con bajo peso al nacimiento, debido a procesos de restricción del crecimiento intrauterino que confirman la importancia del ambiente prenatal. Los lechones de bajo peso al nacimiento se ven afectados por mayores tasas de mortalidad y por alteraciones en su desarrollo y su metabolismo postnatal que pueden afectar a la calidad de la carne y la canal. Todos estos procesos son perjudiciales para la rentabilidad de la explotación porcina.

Los efectos de la variabilidad intracamada y el bajo peso al nacimiento no han sido estudiados en profundidad en cerdos de razas tradicionales, como el cerdo ibérico. El cerdo ibérico es originario de la Península Ibérica y es especialmente valorado por sus productos cárnicos de alta calidad. Esta raza de cerdo graso ha sido escasamente seleccionada y se ha criado en condiciones extensivas durante siglos, por lo que presenta menores rendimientos productivos y reproductivos y un mayor problema de heterogeneidad que las razas magras, así como una diferente fisiología que caracteriza y condiciona su producción.

Por ello, el objetivo principal de esta tesis doctoral ha sido describir y caracterizar, en la raza ibérica, los efectos postnatales del peso al nacimiento hasta el sacrificio y determinar su efecto causal en la variabilidad de los lotes de producción. Estos resultados establecen las bases para el diseño de posibles estrategias, principalmente nutricionales, encaminadas a mejorar su productividad y rentabilidad.

Para ello se realizaron tres diseños experimentales. Los experimentos 1 y 2 fueron realizados en condiciones de granja comercial. El primer experimento tenía como objetivo la valoración de la variabilidad del peso al nacimiento, de la incidencia de lechones de bajo peso al nacimiento en la raza ibérica y de los efectos de esta variabilidad sobre el crecimiento postnatal y sobre la calidad de la canal y la carne. El segundo experimento tenía como objetivo el estudio del efecto de una restricción alimentaria materna ligera durante la gestación sobre las variables anteriores. Por último, el tercer experimento se realizó en condiciones de granja experimental para evaluar la utilidad de la suplementación materna

durante la gestación con hidroxitirosol, un polifenol del olivo, para la prevención de los efectos negativos de la restricción de crecimiento intrauterino en el cerdo ibérico.

Los resultados de los experimentos 1 y 2 mostraron que, como en otras razas, el aumento del tamaño de camada en el cerdo ibérico incrementa la cantidad de lechones de bajo peso al nacimiento y la variabilidad del peso al nacimiento. Además, la disminución del peso al nacimiento medio de los lechones como consecuencia de una mayor prolificidad es más intensa en la raza ibérica que en las razas genéticamente seleccionadas. Un menor peso al nacimiento generó un crecimiento postnatal más lento y menos eficiente, lo que aumentó la edad a matadero. Además, se produjo una disminución de la calidad de la canal y de la carne y alteraciones metabólicas relacionadas con fenómenos de engrasamiento y resistencia a la insulina. Estos efectos estuvieron modulados por el sexo. En el inicio de la fase de crecimiento, los animales de menor peso al nacimiento tuvieron un crecimiento compensatorio que fue sobre todo beneficioso para las hembras. Sin embargo, el crecimiento posterior de las hembras de menos de 1 kg de peso al nacimiento se enlenteció y no consiguieron mantener el ritmo de crecimiento esperado.

El efecto del peso al nacimiento se mantuvo durante todo el periodo postnatal y el producto final, pero resultó ser mayor en el inicio de la vida postnatal; así, las alteraciones en la calidad de la canal y de la carne fueron menores de las esperadas. Además, los datos obtenidos sugieren que se debería establecer el valor de 1 kg de peso al nacimiento para el triaje de los lechones al nacimiento. De este modo, se podrían usar estrategias nutricionales y de manejo específicas que mejoren su desarrollo y evitar su uso como animales reproductores por posibles alteraciones transgeneracionales.

Los datos obtenidos en el segundo experimento desaconsejan el uso de una ligera restricción de la alimentación materna antes del periodo de máximo crecimiento del feto como estrategia nutricional para ahorrar costes. Aunque no se observan diferencias al nacimiento o al destete respecto a los lechones procedentes de camadas sin restricción, los lechones procedentes de cerdas restringidas presentaron peores índices de crecimiento y trastornos metabólicos, con una peor calidad de la canal y de la carne; lo que puede penalizar finalmente la rentabilidad de la explotación.

Por último, el tercer experimento ha demostrado el efecto positivo en la raza ibérica de la suplementación materna de hidroxitirosol a 1.5 mg/día durante la gestación en el desarrollo prenatal y postnatal temprano. Los lechones procedentes de madres tratadas mostraron mayores pesos al nacimiento y una menor incidencia de lechones inferiores a 1 kg. Estos efectos fueron especialmente beneficiosos en las camadas de mayor número de

lechones, en principio los más perjudicados por la restricción de crecimiento intrauterino, ya que favoreció un mayor crecimiento durante la lactación en los lechones tratados respecto a los controles.

La información obtenida en el desarrollo experimental de esta tesis doctoral muestra, en resumen, la importancia del peso al nacimiento sobre la variabilidad de los lotes productivos en el cerdo ibérico y señala la existencia de diferencias respecto a lo descrito previamente en razas magras. Además, aporta una base de conocimiento para el desarrollo de posibles estrategias nutricionales y de manejo que permitan disminuir y gestionar esa variabilidad en la producción comercial de cerdo ibérico, y, así, optimizar la rentabilidad del sector.

# Abstract

The optimization of profitability in swine production is mainly based on the increase in the kg of meat produced per sow and year and on the homogeneity in the growth and slaughter batches. Thus, in genetically selected commercial breeds, the increase in the number of piglets per litter has been sought. However, litters with a high number of piglets are penalized by increases in birth-weight variability and by a higher percentage of piglets with low birth-weight, due to intrauterine growth restriction processes, which confirm the importance of the prenatal environment. Low birth-weight piglets are affected by higher mortality rates and by alterations in their development and postnatal metabolism, which can affect the carcass and meat quality. All these processes are harmful to the pig farm profitability.

The effects of within-litter variability and low birth-weight have never been studied in traditional pig breeds, such as the Iberian pig. The Iberian pig is native to the Iberian Peninsula and especially valued for its high-quality meat products. This fatty pig breed has been scarcely selected and bred in extensive conditions for centuries, showing lower productive and reproductive yields and a greater heterogeneity than lean breeds, as well as different physiological features that characterizes and determines its production.

Therefore, the primary objective of this Doctoral Thesis was to describe and characterize, in the Iberian breed, the postnatal effects of birth-weight until the slaughter and to determine its causal effect on the variability in the production batches. Results provide the basis for the design of possible strategies, mainly nutritional, aimed at improving their productivity and profitability.

For this purpose, three experiments were designed. Experiments 1 and 2 were carried out under commercial farm conditions. The first experiment aimed to obtain information regarding the birth-weight variability and the incidence of low birth-weight piglets in the Iberian breed and the effects of this variability on the postnatal growth and the carcass and meat quality. The second experiment aimed to study the impact of a slight maternal dietary restriction during pregnancy on the above variables. Finally, the third experiment was carried out under experimental farm conditions to evaluate the usefulness of maternal supplementation during pregnancy with hydroxytyrosol, an olive polyphenol, for the prevention of the adverse effects of intrauterine growth restriction in the Iberian pig.

The results of experiments 1 and 2 showed that, like in other breeds, the increase in litter size in the Iberian pig increases the number of low birth-weight piglets and the variability of birth-weight. In addition, the decrease in the piglet average birth-weight as a consequence of a greater prolificacy is more intense in the Iberian breed than in the genetically selected breeds. Lower birth-weight pigs had slower and less efficient postnatal growth, which increased the age of slaughter. There was also a decrease in the carcass and meat quality and metabolic alterations related to fattening and insulin resistance in low birth-weight pigs. These effects were modulated by sex. At the beginning of the growing phase, lower birth-weight pigs had a compensatory growth that was especially beneficial for females. However, the subsequent growth of females with birth-weight lower than 1 kg decreased and they failed to maintain the expected growth rate.

The effect of birth-weight was maintained throughout the postnatal period and the final product, but it was higher at the beginning of postnatal life; thus, the alterations in the carcass and meat quality were lower than expected. In addition, the data obtained suggest that a value of 1 kg of birth-weight should be established for the triage of piglets at birth. In this way, specific nutritional and management strategies could be used to improve their development and avoid their use as breeding animals due to possible transgenerational alterations.

The data obtained in the second experiment discourage the use of a slight nutritional restriction of pregnant sows before the stages of final maximum fetal growth as a nutritional strategy to save costs. Although no differences were observed between piglets from restricted litters and control litters either at birth or at weaning, piglets from restricted sows showed poorer growth rates and metabolic disorders, with a worse carcass and meat quality. These features can finally penalize farm profitability.

Finally, the third experiment demonstrated the positive effect, in the Iberian breed, of maternal hydroxytyrosol supplementation with 1.5 mg/day during gestation on prenatal and early postnatal development. Piglets from treated sows showed higher birth-weights and a lower incidence of piglets with birth-weight lower than 1 kg. These effects were especially beneficial in the largest litters, which are the most harmed by the intrauterine growth restriction, because of a greater growth during lactation of treated than of control piglets.

The data obtained in the experimental procedures of the presents Doctoral Thesis show, in summary, the importance of birth-weight on the variability of productive batches in the

Iberian pig and points out the existence of differences with regards to previously described in lean breeds. It also provides a base of knowledge for further development of nutritional and management strategies aiming to reduce and manage this variability in the commercial production of Iberian pigs and therefore boosting profitability.

# 1. Introducción y objetivos

España es el cuarto país productor de carne de cerdo a nivel mundial, destacando la producción de productos cárnicos curados por su alto valor añadido y principalmente aquellos procedentes del cerdo ibérico, por sus características organolépticas de calidad valoradas a nivel nacional e internacional.

El cerdo ibérico es un cerdo graso autóctono de la Península Ibérica que pese a su interés económico, presenta menores rendimientos productivos y reproductivos que razas más seleccionadas. Una de sus principales limitaciones es una menor prolificidad en comparación con otras razas comerciales, lo que es negativo para la rentabilidad de una explotación (Lopez-Bote 1998). Por ello, para aumentar el beneficio económico es crucial aumentar este parámetro y poder producir más cerdos por cerda y año. Esto implica no sólo un aumento de la prolificidad, sino también una adecuada viabilidad de la progenie y mayores índices de crecimiento a lo largo del ciclo productivo. Al mismo tiempo, se busca uniformidad en los lotes a matadero para disminuir los costes de producción y para facilitar los procesos de elaboración de productos en la industria cárnica.

En razas magras en las que se ha incrementado la prolificidad, este aumento se traduce en un menor peso al nacimiento de los lechones y una mayor heterogeneidad de pesos intracamada; que se ha relacionado con fenómenos de retraso del crecimiento intrauterino por falta de espacio uterino (Wu *et al.* 2006). La restricción de crecimiento intrauterino causa lechones de bajo peso al nacimiento, lo que puede condicionar su supervivencia y evolución postnatal (Quiniou *et al.* 2002). Esto afecta a la productividad final de la explotación al influir sobre los kilos de carne producidos por cerda y la homogeneidad de los lotes de sacrificio.

En la actualidad, para disminuir las consecuencias negativas de la restricción de crecimiento intrauterino, se trabaja con estrategias nutricionales en las reproductoras basadas en distintos niveles de aporte y en la inclusión de nutrientes que mejoren el crecimiento prenatal (Wu et al. 2010; Mordhorst and Prather 2017). En los últimos años, estos estudios se están dirigiendo hacia grupos de sustancias antioxidantes como los polifenoles.

A pesar de la importancia de la raza ibérica dentro de nuestro sector porcino, hay escasez de información sobre la variabilidad del peso al nacimiento y sus efectos postnatales. En este sentido, estudios anteriores de nuestro grupo señalan su influencia sobre los rendimientos productivos y la importancia de factores como la nutrición materna durante la gestación y el sexo del lechón y su manejo durante el periodo de

crecimiento (Gonzalez-Bulnes *et al.* 2012a; Barbero *et al.* 2013; Barbero *et al.* 2014). Tampoco se dispone de información relativa al efecto de estrategias nutricionales prenatales como el aporte de polifenoles en la dieta materna. Por ello, en el cerdo ibérico, el estudio del efecto que el peso al nacimiento tiene a nivel postnatal y el desarrollo de posibles estrategias nutricionales para disminuir su variabilidad se presentan como un campo de investigación de gran relevancia.

En base a estas consideraciones, el objetivo general de esta tesis doctoral es la obtención de información sobre la variabilidad del peso al nacimiento y sus efectos en cerdo ibérico, así como el diseño de posibles estrategias para mejorar su productividad y rentabilidad.

Para ello, los objetivos específicos de esta tesis doctoral son:

1. Estudiar la variabilidad del peso al nacimiento, su variabilidad intra- e intercamada y la influencia del sexo en el cerdo ibérico en condiciones de granja.

2. Estudiar los efectos del sexo y el peso al nacimiento, y su variabilidad intracamada, sobre la supervivencia y el crecimiento postnatales y los parámetros de calidad de la canal y de la carne en el cerdo ibérico condiciones de granja.

3. Conocer el efecto de una restricción nutricional materna ligera sobre la aparición de lechones de bajo peso al nacimiento y su efecto posterior sobre la supervivencia, el crecimiento postnatal y los parámetros de calidad de la canal y de la carne en el cerdo ibérico en condiciones de granja, valorando el efecto del sexo.

4. Valorar el efecto del uso de un polifenol, el hidroxitirosol, como estrategia nutricional prenatal para reducir la variabilidad del peso al nacimiento y mejorar el crecimiento hasta el destete en el cerdo ibérico, valorando el efecto del sexo.

# 2. Antecedentes y estado actual

El ganado porcino en España representa el 12.7% de la Producción Final Agraria y ocupa el primer puesto de la Producción Final Ganadera (36.4% del total) con un valor anual de más de 4.000 millones € anuales (MAPAMA 2017b). Actualmente, España es el cuarto productor de carne de cerdo a nivel mundial y el segundo dentro de la Unión Europea (UE) con 4.3 millones de toneladas de carne procedentes de casi 50 millones de cerdos sacrificados (**Figura 2.1.**; CE 2018). Además, es el país con el mayor censo comunitario con 30 millones de cerdos (Eurostat).

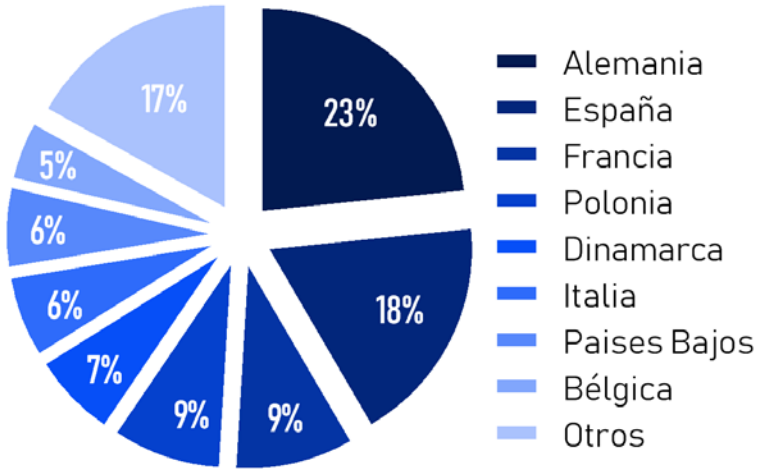


**Figura 2.1.** Producción de toneladas de carne de cerdo por países dentro de la Unión Europea (Eurostat).

En los últimos años el sector porcino español ha ido aumentando tanto a nivel censal como de producción cárnica gracias a dos factores principales. Por un lado, el aumento del autoabastecimiento en España, que alcanza el 174% y en el que destaca el consumo de productos trasformados (MAPAMA 2017b). Y por otro, la internacionalización del comercio y el aumento de las exportaciones, que suponen el 45% de la producción. El principal importador es la UE con 2 millones de toneladas (60% del total; MAPAMA 2017b).

A nivel internacional, la UE es la principal potencia exportadora de productos derivados del cerdo y, en el año 2017, España ha sido el segundo exportador europeo en toneladas de carne con unas ganancias económicas de 1675 millones de Euros (CE 2018). Dentro de las exportaciones, destacan los preparados cárnicos, en especial las carnes saladas, por ser productos de alto valor añadido en comparación a otras categorías de productos (MAPAMA 2017b; CE 2018). En este sentido, la exportación de carnes saladas, como son los jamones y las paletas curadas, generó ingresos cercanos a 400 millones de Euros en el 2017 (ICEX 2017). En esta categoría de preparados cárnicos destacan los procedentes de cerdo ibérico por su alto valor económico (MAPAMA 2017a).

La comercialización de estos productos curados en el subsector del cerdo ibérico se encuentra reglada por la Norma de Calidad para la carne y productos cárnicos ibéricos del vigente Real Decreto 4/2014 (Ministerio de Agricultura 2014). Según dicha normativa, los cerdos se clasifican dependiendo de la pureza racial (si hay cruce o no con machos Duroc) y del sistema de explotación y alimentación. Este sistema marca las cuatro denominaciones de venta aceptadas: de bellota 100% ibérico, de bellota ibérico, de cebo de campo ibérico y de cebo ibérico.

En resumen, a nivel racial, los cerdos son 100% ibérico cuando el padre y la madre son ibéricos puros, pueden ser también 50% o 75% ibérico cuando el padre es de genética Duroc o 50% ibérico, respectivamente. Respecto a nivel productivo, la categoría de bellota se otorga por crianza en montanera sin pienso en los últimos meses de vida, la de cebo de campo por crianza en extensivo o intensivo al aire libre con piensos y la de cebo por crianza en intensivo.

Cada año, el número de cerdos ibéricos sacrificados y de piezas, como los perniles para la producción de jamones curados, aumenta en cada una de las categorías anteriores (**Tabla 2.1.**: ASICI ; MAPAMA 2017b). La categoría de mayor volumen es la de cebo con cerca del 60% de los cerdos ibéricos sacrificados, siendo en su mayoría del cruce 50% ibérico.

**Tabla 2.1.** Número de precintos de la Norma de Calidad en Jamones por categorías de venta entre 2014 y 2017 (ASICI).

| Año | IB Bell 100% | IB Bell | IB CC | IB Cebo | IB Total |
|---|---|---|---|---|---|
| 2014 | 296,227 | 553,954 | 643,79 | 3,289,383 | 4,783,354 |
| 2015 | 412,678 | 567,145 | 1,155,473 | 3,456,442 | 5,591,738 |
| 2016 | 513,734 | 621,485 | 1,241,492 | 3,728,388 | 6,105,099 |
| 2017 | 594,868 | 675,345 | 1,327,547 | 3,882,559 | 6,480,319 |

IB: ibérico; Bell: Bellota; CC: Cebo de campo

## 2.1. El cerdo ibérico y sus características diferenciales

El cerdo ibérico es una raza evolucionada a partir del *Sus scrofa mediterraneus* y la única Raza Autóctona Porcina de Fomento de España, dado que es la única raza originaria que se produce industrialmente, aunque no todas sus variedades se encuentran en esta categoría (M. Aparicio 1988; MAPAMA 2008). A pesar de su explotación industrial, es una raza tradicional y sus parámetros productivos, reproductivos y rendimientos de la canal se consideran peores que los obtenidos en razas más seleccionadas (Lopez-Bote 1998). Dentro de Europa, hay dos razas tradicionales que se utilizan para la elaboración de productos cárnicos, principalmente chacineros, a nivel industrial, que son la raza Mangalica en Hungría y la raza ibérica en España y Portugal. Entre ellas, el cerdo ibérico destaca por su censo y su producción de productos de calidad reconocidos a nivel internacional por sus características organolépticas de alta calidad, principalmente ligadas a su textura, color, sabor, aroma y jugosidad (Serra *et al.* 1998; Nieto *et al.* 2002).

En su conjunto, la raza ibérica representa casi un 11% del censo porcino nacional, con un aumento del 3% sobre el censo nacional en los últimos cuatro años (**Tabla 2.2.**; MAPAMA 2017b). Además, también el número de reproductoras (cerdas 100% ibéricas) inscritas en el ITACA (el sistema de control del cerdo ibérico) también ha crecido hasta superar actualmente las 350 mil cerdas (ASICI).

**Tabla 2.2.** Censo del porcino ibérico en España por Comunidades Autónomas en noviembre del año 2013 y del año 2017 (MAPAMA 2017b).

| Comunidades Autónomas | 2013 | 2017 |
|---|---|---|
| Castilla y León | 678,077 | 886,670 |
| Castilla la Mancha | 67,549 | 200,999 |
| Extremadura | 1,014,489 | 1,267,327 |
| Andalucía | 591,193 | 893,531 |
| Total | 2,351,566 | 3,248,777 |

### 2.1.1. Sistemas de producción

Tradicionalmente, esta raza singular y su producción han estado asociadas a un sistema de crianza extensivo o semi-extensivo de baja productividad en el ecosistema de la Dehesa, situado en el suroeste de la península ibérica (**Figura 2.2.**), Este bosque

mediterráneo se conserva gracias a la actividad del ser humano como un sistema agrosilvopastoral en el que la ganadería, destacando el cerdo ibérico, es primordial. Además, las actividades en ella desarrolladas permiten el mantenimiento de la actividad socioeconómica en las zonas rurales (Lopez-Bote 1998).

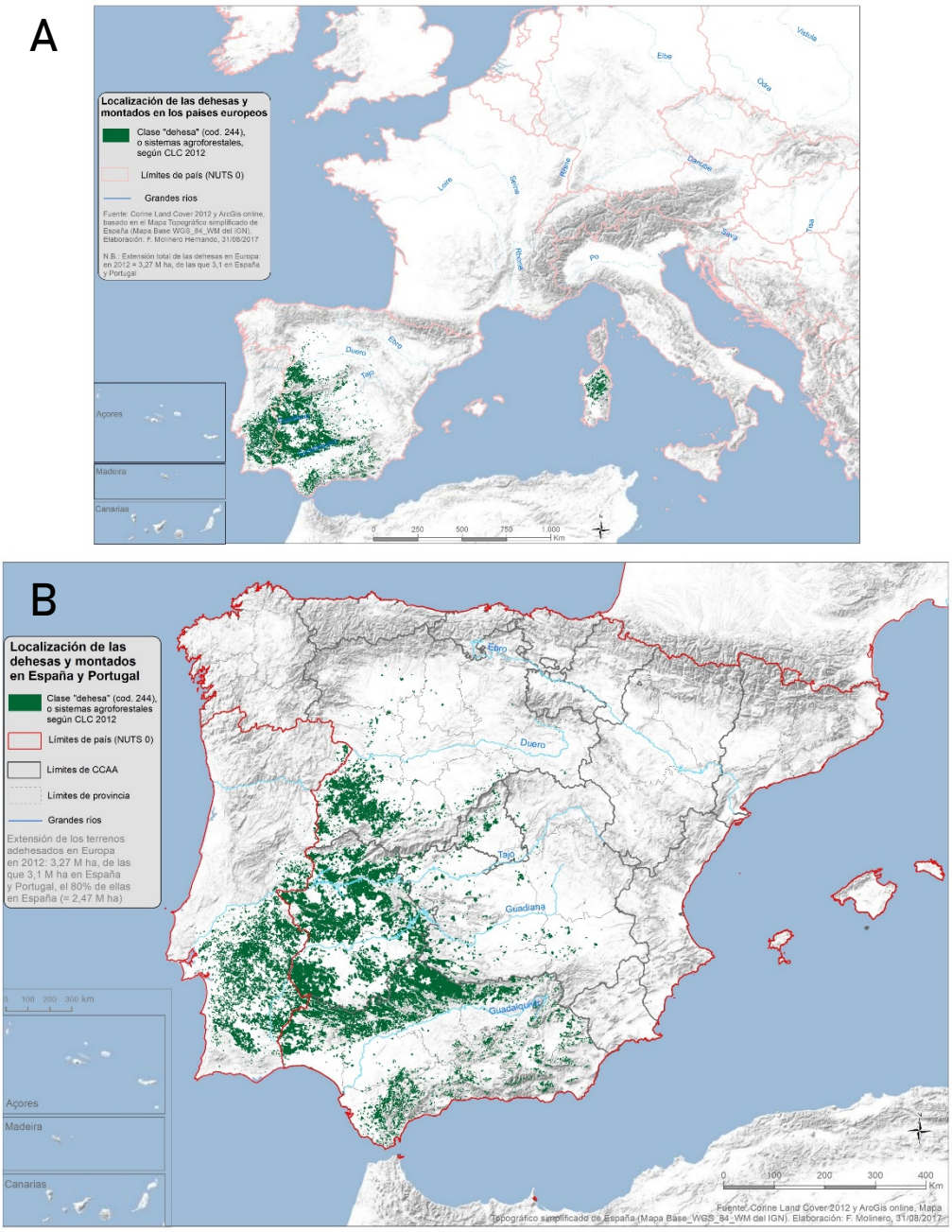


**Figura 2.2.** Distribución geográfica del ecosistema de dehesa (Molinero 2017). A: Localización de las dehesas y montados en Europa. B: Localización de las dehesas y montados en España y Portugal.

El uso del sistema de producción intensiva aumenta la rentabilidad del cerdo ibérico perdiendo la temporalidad que marca la crianza de los cerdos ibéricos mediante el sistema en extensivo o semiextensivo y permite acortar los ciclos de producción (Buxadé Carbó and Daza Andrada 2001).

La diferencia más evidente al comparar con el sistema en intensivo para otras razas de cerdos es la gran duración del ciclo productivo, ya que los animales deben tener un mínimo de 10 meses de vida y entre 140-160 kg de peso vivo (PV) para su sacrificio. Este hecho condiciona el manejo de los animales para evitar la presencia de olor sexual en la carne, por tanto se realiza la castración de los machos de forma quirúrgica o mediante (Weiler *et al.* 1997; Meier-Dinkel *et al.* 2013).

Debido a la duración de su ciclo productivo, algunas de las fases en el sistema intensivo y su alimentación tienen diferencias respecto a otras razas. Las primeras fases de vida englobarían la fase de lactación y la fase de transición, que son muy similares a otras razas. La fase de lactación comienza tras el parto y se extiende hasta los 21-28 días de vida del lechón. El momento del cambio a la siguiente fase se produce con el destete, en el que los lechones serán separados de sus madres y pasarán a estar en cuadras o parques junto con lechones de su propio lote. En la fase de transición, los lechones permanecen hasta alcanzar los 20-25 kg PV, en torno a los 70-80 días. Tras este periodo, los cerdos entran en la fase de crecimiento hasta alcanzar los 90 kg finalizando sobre los 190 días. Por último, está la fase de cebo que dura hasta que alcanzan su peso de sacrificio entre 145-160 kg PV a los 10 meses. Desde el final del periodo de transición se realiza un racionamiento entre el 70 y 85% de su consumo *ad libitum* para que no alcancen el peso a matadero antes del tiempo marcado por el RD 4/2014. En el caso de genéticas magras los animales alcanzan un peso de 100 kg sobre los cinco meses y medio de vida sin restricciones en la cantidad de pienso que reciben.

### 2.1.2. Particularidades fisiológicas y de calidad de carne

A diferencia de la mayoría de razas porcinas empleadas en la producción actual, el cerdo ibérico es un cerdo graso con gran capacidad adipogénica, gran apetito, crecimiento lento y gran rusticidad (Andrés et al. 1998). La raza ibérica acumula grasa durante las épocas de abundancia para sobrevivir a los periodos de escasez de alimentos en la naturaleza, mediante unas adaptaciones genéticas que conforman un *genotipo ahorrador* (Neel 1962; Gonzalez-Bulnes *et al.* 2016a). Dentro de estas adaptaciones está

descrito un polimorfismo en el gen del receptor de la leptina (LEPR c.1987C/T; Óvilo *et al.* 2005), que contribuiría a su elevada capacidad de consumo y engrasamiento al alterar la señalización de la leptina; una hormona que regula el balance energético a largo plazo, influyendo a nivel hipotalámico sobre el control del apetito (Zhang *et al.* 1994; Klok *et al.* 2006). El cerdo ibérico también se caracteriza por altos niveles de esta hormona en sangre, altos niveles de expresión de ARNm en tejido adiposo y bajos niveles de expresión de su receptor en el hipotálamo comparando con cerdos de genéticas magras (Fernández-Fígares *et al.* 2007; Óvilo *et al.* 2010; Benítez *et al.* 2018). Todas estas características concuerdan con una menor señalización de la leptina en esta raza y con el síndrome de resistencia a la leptina (Myers et al. 2008).

Su alta capacidad adipogénica junto con una menor acumulación de proteína se relacionan con un crecimiento lento y con mayores índices de conversión (IC; Nieto et al. 2002; Barea et al. 2007; Nieto et al. 2012). Sin embargo, no se ha encontrado que la menor acumulación de proteína en el cerdo ibérico se deba a una menor tasa de síntesis proteica (Rivera-Ferre et al. 2005), pero se han descrito cambios en la expresión génica de rutas metabólicas asociadas a una mayor degradación proteica durante fases de crecimiento en cerdos ibéricos, que conducirían a un balance reducido de síntesis proteica (Ovilo *et al.* 2014b). Por otra parte, a los cuatro meses de edad, se ha observado que cerdos ibéricos puros presentan una mayor expresión de rutas metabólicas relacionadas con el desarrollo muscular que cerdos ibéricos cruzados con Duroc. Este cambio coincide con un crecimiento compensatorio, alcanzando el peso de cerdos ibéricos 50%, a pesar del menor peso al nacimiento (PN) (Ayuso et al. 2016). Este crecimiento compensatorio podría verse favorecido por el *genotipo ahorrador* en condiciones de disponibilidad de alimento.

#### *2.1.2.2. Importancia de la grasa intramuscular y la composición en ácidos grasos*

Grasa Intramuscular

Las características del metabolismo del cerdo ibérico, como se ha descrito anteriormente, facilitan la acumulación grasa subcutánea, visceral e intramuscular (**Tabla 2.3.**). Tanto la GIM como la grasa intermuscular representan en torno a un 20-35% de los depósitos grasos, identificándose en pequeñas vacuolas y en las membranas (Gerbens 2004). La GIM se relaciona positivamente con la calidad de la carne, ya que enriquece sus propiedades sensoriales aumentando su aceptación por parte de los consumidores (Fernandez *et al.* 1999b).

Sin embargo, el efecto del contenido en GIM sobre la calidad no es sistemático y puede depender del genotipo. En el caso de cerdos de la raza Duroc, el incremento de la GIM por el aumento de los triglicéridos en la dieta originó un mayor veteado y sabor en la carne; propiedades que no mejoraron en otros cruces raciales (Fernandez *et al.* 1999a). La GIM puede verse también afectada por otros diversos factores como la genética, los cambios epigenéticos, la edad, las hormonas sexuales, el sistema de producción y la alimentación (Ruiz *et al.* 1998; Mayoral *et al.* 1999; Andrés *et al.* 2001; Serrano *et al.* 2008; Bosch *et al.* 2012; Barbero *et al.* 2013). La alimentación puede influir, ya que por ejemplo un aumento de energía puede producir un aumento en la grasa subcutánea pero no necesariamente en la GIM; sin embargo, una disminución de la proteína de la dieta aumenta este parámetro (Barbero Fernández 2015). También el perfil de ácidos grasos (AG) de la dieta y el contenido en micronutrientes pueden afectar la cantidad de GIM en los cerdos (Madsen *et al.* 1992; Flachowsky *et al.* 2008; Lopez-Bote *et al.* 2008; Olivares *et al.* 2009).

**Tabla 2.3.** Revisión del contenido de grasa intramuscular (GIM) en carne fresca.

| Raza y Pureza[1] | Sexo | Pieza | GIM (%) | Detalles | Referencia |
|---|---|---|---|---|---|
| **Cerdo ibérico** | | | | | |
| Sexo | | | | | |
| 50% | ♂ C | L *dorsi* | 8.60 | | (Serrano *et al.* 2008) |
| | ♀ | | 6.10 | | |
| 100% | ♀ QC | *L thoracis* | 9.50 | | (Martinez-Macipe *et al.* 2016) |
| | ♀ | | 8.40 | | |
| | ♀ IC | | 7.70 | | |
| | ♂ QC | | 9.10 | | |
| | ♂ IC | | 7.00 | | |
| 50% | ♂ C | *L thoracis* | 5.99 | | (Egea *et al.* 2016) |
| | ♀ | | 5.01 | | |
| Crianza | | | | | |
| 100% | - | *Biceps femoris* | 8.67 | Cría en exterior | (Andrés *et al.* 2001) |
| 50% | | | 7.99 | | |
| 100% | | | 6.35 | Cría en interior | |
| 50% | | | 5.76 | | |
| 50% | ns | L *dorsi* | 5.87 | Duroc tipo 1 | |

| Raza y Pureza[1] | Sexo | Pieza | GIM (%) | Detalles | Referencia |
|---|---|---|---|---|---|
| | | | 3.32 | Duroc tipo 2 | (Ramírez *et al.* 2007) |
| 100% | ♀ | L *dorsi* | 19.4 MS<br>25.1 MS<br>24.2 MS<br>15.4 MS | Control<br>Sobrenutrición materna toda la gestación<br>Res materna toda la gestación<br>Res materna tardía | (Barbero *et al.* 2013) |
| 50% | ♀ | L *dorsi* | 5.80<br>5.30<br>6.50 | Pienso con C18:2n-6 8.7 g/kg<br>13.5 g/kg<br>16.7 g/kg | (Isabel *et al.* 2014) |
| 100% | ♂ C | *Semimembranosus* | 4.60<br>6.78<br>5.59 | Control<br>Res vit A temprana<br>Res vit A tardía | (Ayuso *et al.* 2015a) |
| | | | Genética | | |
| 100%<br>50% | ♂ | L *dorsi* | 6.07<br>4.27 | | (Ovilo *et al.* 2014b) |
| 100%<br>50% | ♂ | *Biceps femoris* | 2.21<br>1.72 | | (Ayuso *et al.* 2015b) |
| 100%<br>50% | ♂ C | *L dorsi* | 4.05<br>2.87 | | (Ayuso *et al.* 2016) |
| | | | **Cerdo de genética magra** | | |
| | | | Sexo | | |
| (LxLW)x(PxLW) /Danbred | ♂ C<br>♀ | *L dorsi* | 3.40<br>2.70 | | (Latorre *et al.* 2003) |
| Dx(LxLW) | ♀<br>♀ IC | *L thoracis* | 4.26<br>4.99 | | (Daza *et al.* 2014) |
| (DxLxLW)x PIC | ♀<br>♂ | *L dorsi* | 7.47<br>8.24 | | (Segura *et al.* 2015) |
| | | | Crianza | | |
| Cruces D | ns | *L dorsi* | 1.41<br>1.29<br>1.08 | Pienso con sebo<br>oliva<br>soja | (Flachowsky *et al.* 2008) |

| Raza y Pureza[1] | Sexo | Pieza | GIM (%) | Detalles | Referencia |
|---|---|---|---|---|---|
| | | | 1.36 | linaza | |
| D | ♀ | *L dorsi* | 3.60 | Pienso con AGS | (Olivares *et al.* 2009) |
| D | | | 3.60 | AGPI | |
| LxLW | | | 3.00 | AGS | |
| LxLW | | | 2.70 | AGPI | |
| Edad de sacrificio | | | | | |
| D | ♂ | *L dorsi* | 2.78 | 160 días de edad | (Bosch *et al.* 2012) |
| | | | 3.24 | 185 días | |
| | | | 3.91 | 220 días | |

[1] 100%: Ibérico 100%; 50%: Ibérico 50%; LxLW: Landrace×Large White; D: Duroc; P: Pietrain
♂: Macho; ♀: Hembra; C: castrado/a; QC: Quirúrgicamente C; IC: Inmunológicamente C
MS: Materia Seca; L: *Longissimus*; ns: no significativo; Res: Restricción; vit: Vitamina
AGS: 'Ácidos grasos saturado. AGPI: Ácidos grasos poliinsaturados.

Composición en Ácidos Grasos

El perfil de AG de la carne es importante a nivel tecnológico ya que puede estar ligado a problemas de oxidación lipídica, migración de agua o textura de la grasa. Además, se relaciona con la correcta maduración, sabor y olor de los productos cárnicos (Chizzolini *et al.* 1998; Lopez-Bote 1998). El perfil de AG está principalmente determinado por la dieta mediante la acumulación directa de la grasa de esta; sin embargo, factores como la genética, los cambios epigenéticos, la edad, las hormonas sexuales también pueden afectar (Andrés *et al.* 2001; Daza *et al.* 2005; Ventanas *et al.* 2007; Serrano *et al.* 2009; Bosch *et al.* 2012; Barbero *et al.* 2013). Por ejemplo durante la fase de cebo en el cerdo ibérico, se puede incrementar la concentración de ácido oleico (C18:1n-9) en las posiciones externas de los triglicérido de la grasa subcutánea mediante la alimentación en montanera o con piensos con alto contenido en dicho AG, reduciendo la dureza, la adhesividad y el punto de fusión de este tejido especialmente en los cerdos de montanera (Segura 2015). El cerdo ibérico presenta un perfil característico con una mayor proporción de AG monoinsaturados, destacando el C18:1n-9, y con una menor cantidad de AG poliinsaturados que en las razas magras (Serra *et al.* 1998; López-Bote *et al.* 2000; Barea *et al.* 2013). Entre los AG poliinsaturados destaca el Ácido linoleico (18:2n-6) que es un AG poliinsaturado esencial, es decir, sólo se obtiene a través de la dieta. Este AG juega un papel esencial en la consistencia de la grasa y, en el cerdo ibérico, su acumulación está relacionada tanto con su consumo como con la capacidad de utilizarlo a través de la beta-

oxidación (Isabel *et al.* 2014). Para explicar las diferencias entre razas, se ha sugerido que la raza ibérica tiene una mayor síntesis de lípidos *de novo* y una mayor capacidad de desaturación que requiere un mayor coste energético; esto ayudaría a explicar su menor eficiencia energética (Barea *et al.* 2013). Por otro lado, se ha comprobado una mayor expresión de enzimas lipogénicas y desaturasas musculares en ibéricos 100% a distintas edades (Ovilo *et al.* 2014b; Ayuso *et al.* 2016).

Los AG también tienen gran influencia en la nutrición humana. Los nutricionistas recomiendan una alimentación en la que el ratio de AG poliinsaturados n-6/n-3 sea lo más bajo posible (cerca de 4:1) para disminuir el riesgo de algunos cánceres, enfermedades del corazón e incluso de depresiones (Ulbricht and Southgate 1991; Enser *et al.* 2000; Husted and Bouzinova 2016). Dado que la ratio de AG poliinsaturados n-6/n-3 de nuestra dieta oscila entre 20-15:1 cualquier fuente de alimento con AG n-3 que pueda ayudar a disminuirlo siempre será beneficiosa. Los AG poliinsaturados, siempre serán superiores en la fracción de los lípidos polares (FLP), ya que constituyen la membrana celular (los fosfolípidos), que en la fracción neutra de los mismos (FLN). La FLN, que es la fracción mayoritaria a nivel del tejido adiposo subcutáneo y de la GIM con un 70-90%, es la que contiene los depósitos de energía en forma de triacilglicéridos (Wood *et al.* 2008). Principalmente en esta fracción se encuentran los AG monoinsaturados y los AG saturados predominando el C18:1n-9. Según la bibliografía, las dietas ricas en AG monoinsaturados tienen beneficios en la salud de los humanos y disminuyen riesgos metabólicos (Schwingshackl and Hoffmann 2014; Qian *et al.* 2016).

### 2.1.3. La cerda ibérica como reproductora

La suposición tradicional es que la cerda ibérica presenta una menor precocidad reproductiva que razas magras más seleccionadas. Por el contrario, estudios basados en valoraciones hormonales señalan una mayor precocidad en cerdas ibéricas (Gonzalez-Añover *et al.* 2010; Gonzalez-Añover *et al.* 2012). Aproximadamente un 72% de las cerdas ibéricas alcanzaron la pubertad antes de los 180 días de edad, mientras que sólo un 15% de cerdas de genéticas magras alcanzo la pubertad a esa edad (Gonzalez-Añover *et al.* 2010). Los altos niveles de leptina en las cerdas ibéricas están relacionados con su mayor precocidad, ya que una mayor acumulación de tejido adiposo, que segrega leptina, se asocia con la pubertad en varias especies; aunque su peso en el momento de la pubertad fuera menor que el de la raza más seleccionada (92.9 *vs.* 107.8 kg PV). Además, las cerdas

ibéricas presentan un aumento en esta hormona en las semanas anteriores a la pubertad con valores superiores a las cerdas de genéticas magras (Gonzalez-Añover *et al.* 2012).

Otra característica limitante a nivel reproductivo en la cerda ibérica es el aumento del intervalo destete-cubrición, ya que incrementa los días no productivos. En las reproductoras ibéricas este dato es superior al de cerdas de otras genéticas más seleccionadas (8.7 *vs.* 7.6 días; Aparicio *et al.* 2011). Este incremento en la duración del ciclo está relacionado con las repeticiones totales del celo (cerdas que vuelven a salir a celo pasado el periodo normal de cubrición tras el parto) que son un 18.4% de las cerdas cuando el valor límite aceptado como normal es menor al 10%. Las cerdas que más repiten son cerdas que continúan en periodo de crecimiento, encabezando la lista las cerdas de primer parto (28%), seguidas por las de segundo parto (16%) y las nulíparas (15%). Al aumentar el número de parto disminuye la cantidad de repeticiones; sin embargo, los abortos se mantienen estables, aunque en valores altos al comparar con genéticas magras.

Las repeticiones se pueden estudiar por tipos, según el periodo de días en que ocurran tras el celo. Las más abundantes en las cerdas ibéricas son las repeticiones cíclicas, que aparecen siguiendo el ciclo reproductivo de la cerda (más o menos 21 días), que son un 9.6% cuando deberían ser menores al 6%. Este tipo de repeticiones pueden relacionarse con celos silenciosos, fallos en la detección del celo y una mala condición corporal en el momento de la cubrición, Las cerdas ibéricas también presentan altos porcentajes de repeticiones tardías, que ocurren mucho tiempo después de la cubrición entre 45 y 59 días, y de cerdas vacías, cerdas que salen a celo tras el día 60 de gestación. Este tipo de repeticiones suelen estar relacionadas con abortos o fallos en la detección del celo o gestación.

A nivel morfológico y fisiológico, las cerdas ibéricas presentan diferencias respecto a las razas magras. Su útero es de un tamaño más reducido, lo que también ocurre en otra raza tradicional, la raza Mangalica, y por lo tanto su espacio uterino disponible en el momento de la gestación también será menor (**Figura 2.3.**; Brussow *et al.* 2004; Gonzalez-Añover *et al.* 2011b). Por otro lado, el número de mamas totales está entre diez y doce, aunque es difícil que el 100% sean útiles. A su vez, la menor señalización de la leptina podría tener relación con los menores rendimientos reproductivos de esta raza (Torres-Rovira *et al.* 2011). Esto se debe a que la hormona y su receptor, que se expresa en ovario, oviducto y útero, parecen intervenir en la reproducción y, concretamente, en la

implantación y durante las primeras fases del desarrollo embrionario (Kawamura *et al.* 2002; Yoon *et al.* 2005; Przala *et al.* 2006; Arias-Alvarez *et al.* 2010),

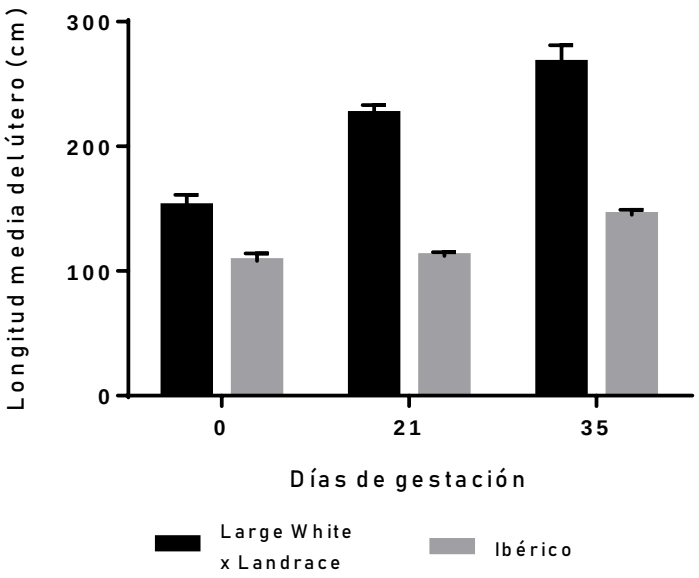


**Figura 2.3.** Cambio en la longitud del útero (cm) en cerdas de genéticas seleccionadas e ibéricas durante el primer trimestre de gestación (Gonzalez-Añover et al. 2011b).

En cuanto a sus datos reproductivos, la media de lechones nacidos totales por parto en cerdas ibéricas, entre seis y siete lechones, es menor al encontrado en otras razas magras con una media de 13.5 lechones, pero los valores de lechones nacidos muertos o momificados son incluso más bajos (Suárez MV 2002; Rueda Sabater 2007; Aparicio *et al.* 2011). Los PN medios también son menores, están entre 1.11 y 1. 37 kg para los lechones ibéricos puros, mientras que en los lechones de genéticas magras la media es en torno a 1.5 kg (Rojas *et al.* 2000; Sánchez-Esquiliche *et al.* 2012). Otro dato menor es la duración media de la gestación, con 112.7 días respecto a las cerdas de otras genéticas con 114.6 días (András *et al.* 2014).

## 2.3. Optimización del rendimiento productivo en el cerdo ibérico

Para incrementar la rentabilidad de la raza ibérica, es necesario aumentar la productividad y disminuir la variabilidad en los lotes de producción; al mismo tiempo que se mejoran sus rendimientos cárnicos y la calidad de sus productos. Para alcanzar estos objetivos se trabaja a través de tres vías principales: la genética, la nutrición y la reproducción.

### 2.3.1. Mejora genética

La finalidad de los programas de mejora genética en especies ganaderas es establecer una presión de selección de alelos favorables para genes que controlan determinados caracteres de interés. Por un lado, estarían los caracteres reproductivos como son la prolificidad (número de lechones nacidos), el número de lechones destetados y la longevidad de la cerda reproductora. Por otro, los caracteres de producción como son el crecimiento en cebo y el rendimiento cárnico. Y por último, los caracteres de calidad como la GIM y el perfil de AG. La mejora se puede realizar a través de la selección genética cuantitativa (por el valor genético mejorante para cada reproductor), molecular (por genotipo) o por cruzamiento (heterosis). Normalmente se combinan las diferentes estrategias. En el cerdo ibérico no hay un gran desarrollo de la mejora genética, pero existe un esquema de selección oficial gestionado por AECERIBER para criterios de selección sencillos como son la ganancia media diaria de peso (GMDP) en cebo y el porcentaje de piezas nobles a matadero; aunque todavía hace falta trabajar en él. A su vez se puede utilizar los cruzamientos entre estirpes de ibérico para mejorar caracteres y es habitual el cruzamiento con la raza Duroc.

Durante mucho tiempo, en los programas de mejora clásica de razas magras, la prioridad han sido el crecimiento y los rendimientos cárnicos dejando de lado los reproductivos, en parte por su baja heredabilidad, y la calidad de carne. En la actualidad, las tecnologías de análisis del genoma a gran escala han experimentado una gran evolución, lo que ha facilitado la disponibilidad de herramientas de análisis muy potentes, Estas herramientas se están empleando activamente para el estudio en profundidad del genoma y su influencia sobre los caracteres de interés económico. Por ello, se considera que los estudios genéticos moleculares pueden aportar soluciones a la mejora de caracteres para los que la selección tradicional no ha resultado eficaz. Existen abundantes estudios moleculares de caracterización de polimorfismos, genes o regiones genómicas asociadas a distintos parámetros productivos en el cerdo ibérico (Ovilo *et al.*

2002; Fernández *et al.* 2012; Ayuso *et al.* 2015b; Ayuso *et al.* 2016; Fernández *et al.* 2017; Martínez-Montes *et al.* 2018). Sin embargo, aunque la investigación es muy intensa, la aplicación práctica de técnicas moleculares es aún escasa en el sector porcino en general y en las razas tradicionales especialmente.

### 2.3.2. Mejora de la nutrición

La alimentación del cerdo ibérico ha experimentado un gran avance en los últimos años, tanto en el manejo de la alimentación como al adecuar las características nutritivas de los piensos a sus necesidades. En la actualidad, hay dos grandes vías de actuación: por un lado, la optimización de las raciones de reproductoras y lechones y, por el otro, la formulación de raciones para crecimiento y cebo que mejoren la calidad de los productos de la raza ibérica, con el objetivo de aproximarse a las cualidades de los productos de cerdos ibéricos criados en extensivo con una alimentación en su etapa final de cebo en base a pasto y bellotas. A lo largo de estos años, el estudio de la fisiología de la raza ibérica ha permitido conocer su menor capacidad de acumulación proteica en comparación con las razas magras y adaptar la formulación de los piensos para las distintas fases productivas, reduciendo la concentración de proteína en las raciones y, por ello, disminuyendo costes y el nivel de nitrógeno en los vertidos (Nieto *et al.* 2002; Barea *et al.* 2007).

Respecto a los suplementos en los piensos de cebo, se intenta imitar las características de la alimentación en la Dehesa que es rica en AG monoinsaturados, vitamina E, AG poliinsaturados n-3 y cobre (Rey *et al.* 1997; Rey and López-Bote 2001; Daza *et al.* 2005). La vitamina E mejora la estabilidad oxidativa de la grasa, lo que es especialmente interesante para el tipo de productos cárnicos que se producen (Isabel *et al.* 1999; Ruiz *et al.* 2005). También reduce las perdidas en el oreo (Isabel *et al.* 2009). Otra vitamina con efecto sobre la calidad de carne es la vitamina A. Su restricción durante la fase de crecimiento y cebo produce un aumento en la GIM y en los AG monoinsaturados de la grasa subcutánea y de la GIM (Ayuso *et al.* 2015a). Esto es positivo porque un perfil con mayor cantidad de AG monoinsaturados mejora la consistencia de la grasa (Isabel *et al.* 2003). La sustitución parcial de C18:2n-6 (AG poliinsaturado) en las dietas mediante el uso de C18:1n-9 (AG monoinsaturado) y vitamina E favorece una menor lipoxidación de la carne, un menor ratio de AG poliinsaturados n-6/n-3 y mejoraba características reológicas como la elasticidad, la cohesividad y la adhesividad (Lopez-Bote *et al.* 2002;

Lopez-Bote *et al.* 2003). Respecto al cobre, se ha visto que la suplementación de los piensos puede incrementar los AG monoinsaturados y disminuir los AG saturados y combinando su uso con vitamina E no aumenta la oxidación lipídica (López Bote and Rey 2001).

### 2.3.2. Mejora de la reproducción

Actualmente, los dos frentes de mayor importancia en las hembras ibéricas son la disminución de los días no productivos y el aumento de la prolificidad. En cuanto a los días improductivos, como se ha comentado anteriormente (*Apartado 2.2.4.*), el inicio de la pubertad no es *a priori* un problema en la cerda ibérica; sin embargo, el alto número de días del intervalo destete cubrición sí. Para disminuir este parámetro, teniendo en cuenta el número de repeticiones se debe mejorar el manejo de la detección de celo, la eficiencia de la inseminación y la nutrición de las reproductoras.

Una mala condición corporal, por exceso o por defecto, puede producir efectos negativos incrementando las repeticiones; por ello, hay que cuidar el estado nutricional de la cerda antes de la gestación (Ashworth *et al.* 2009). Un déficit nutricional en torno a la ovulación, por una alimentación inadecuada durante la lactación, puede generar un incremento del intervalo destete-cubrición y, además, puede afectar, también, a la prolificidad del siguiente parto por descenso de la tasa de ovulación y reducción de supervivencia embrionaria al afectar a la calidad de los ovocitos (Dourmad *et al.* 1994). En lactación, un descenso en el consumo puede generar desórdenes metabólicos como son la resistencia a la insulina y la dislipemia, a los que la cerda ibérica tiene predisposición, que afectarán a su condición corporal tras el destete (Kim *et al.* 2009; Torres-Rovira *et al.* 2011; Gonzalez-Bulnes *et al.* 2016a). Además, si no se solucionan estos problemas, pueden producirse alteraciones de la viabilidad o los patrones de crecimiento de la siguiente progenie por fenómenos de programación prenatal (Gonzalez-Bulnes *et al.* 2012b).

La prolificidad en la cerda ibérica, como en otras especies y razas politocas, puede verse condicionada por la tasa de ovulación y/o la viabilidad embrionaria. La tasa de ovulación no parece ser el mayor condicionante en la cerda ibérica, puesto que según estudios basados en el contaje directo de cuerpos lúteos alcanzan cuotas ovulatorias altas, similares a las de razas más seleccionadas (Gonzalez-Añover *et al.* 2011a; Gonzalez-Añover *et al.* 2011b). Sin embargo, en cerdas con altos niveles de ovulación los embriones viables fueron los mismos que en el caso de cerdas con niveles más bajos

debido a un mayor número de perdidas embrionarias (**Figura 2.4.**; Torres-Rovira *et al.* 2012). Por ello, las pérdidas embrionarias, en estos casos, son el factor condicionante de la prolificidad, ya que un mayor ratio de ovulación por sí sólo no significa el aumento del número de lechones nacidos (Foxcroft *et al.* 2006; Freking *et al.* 2007; Gonzalez-Añover *et al.* 2011b).

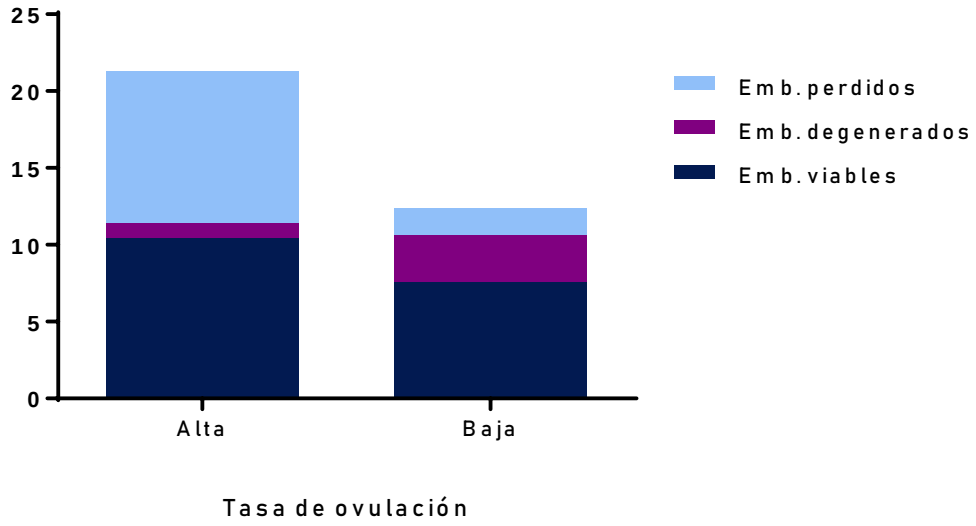


**Figura 2.4.** Número medio de embriones (Emb.) perdidos, viables y degenerados en cerdas ibéricas con alta y baja tasa de ovulación (Torres-Rovira et al. 2012).

Estudios en cerdas Mangalica, que es una raza similar a la raza ibérica, mostraron un menor desarrollo folicular y una maduración intrafolicular más prolongada del ovocito que en cerdas de genéticas seleccionadas, lo que puede afectar a la viabilidad embrionaria (Egerszegi *et al.* 2001). Las pérdidas a nivel embrionario, que principalmente ocurren entre la implantación y el final de la organogénesis, también se ven afectadas por el espacio disponible a nivel uterino, es decir, por la capacidad uterina, que hemos descrito como menor en las cerdas ibéricas (*Apartado 2.1.3.*; Whittemore and Kyriazakis 2006; Van der Waaij *et al.* 2010). Por ello, la disponibilidad de espacio a nivel uterino podría ser el factor más limitante en las cerdas ibéricas. No sólo afectará a nivel embrionario, también puede afectar a nivel fetal mediante la restricción del crecimiento intrauterino (RCIU) en algunos fetos produciendo lechones con bajo PN (BPN). La variabilidad del PN, especialmente el BPN, puede repercutir en la viabilidad y desarrollo postnatal de la progenie disminuyendo la homogeneidad en la camada (Wu et al. 2006). Sin embargo, la mayoría de los datos disponibles se han obtenido en razas magras y la información sobe el desarrollo postnatal en el cerdo ibérico es escasa.

### *2.3.3.1. Influencias del periodo prenatal en el peso al nacimiento*

La cerda al ser una hembra politoca va a gestar un gran número de fetos. Para su adecuado desarrollo, los embriones requerirán espacio para implantarse y después nutrientes y oxigeno suministrados por la cerda a través de la placenta. Por ello, el desarrollo prenatal se ve afectado por la capacidad uterina, la eficiencia placentaria y la alimentación de la cerda; que serán descritos en este apartado.

Hay otros factores que afectan, también, a las reproductoras y pueden influir a nivel de la progenie como son los factores intrínsecos de la cerda y los factores ambientales. Entre los factores intrínsecos se encuentran la raza, ya que pueden tener diferentes estrategias reproductivas, y el número de parto, obteniéndose más lechones de BPN en cerdas más viejas (Wilson et al. 1998; Père and Etienne 2000; Quesnel et al. 2008; Wientjes et al. 2012). Por otro lado, están los factores ambientales como el estrés por un manejo excesivo o por la jerarquía social y el ambiente en las explotaciones, destacando la temperatura y la ventilación. Cualquier fallo o alteración en los todos factores anteriormente comentados puede generar muertes embrionarias y/o fetales, produciendo reabsorciones o momificaciones, o un proceso de RCIU con diferentes grados de severidad. El cerdo es la especie en la que esto ocurre espontáneamente con mayor frecuencia (Wang *et al.* 2008).

**Capacidad uterina**

Aunque hay estudios que relacionan la calidad de los ovocitos con una mayor uniformidad y menor ocurrencia de RCIU (Lee and Haley 1995), se ha observado que el factor más limitante es el espacio uterino disponible que disminuye al aumentar el tamaño de las camadas y que se relaciona con un menor PN medio (**Figura 2.5.**). Cuando la capacidad uterina no es suficiente se genera mayor variabilidad dentro de la camada con diferencias de peso que irán aumentando a lo largo de la gestación (Vonnahme *et al.* 2002; Vallet *et al.* 2014). Además, el aporte de sangre en el útero, y con ello de nutrientes y oxígeno, también se ha descrito como un factor limitante para mantener un correcto desarrollo de los fetos, ya que en grandes camadas no se incrementa lo suficiente para mantener el mismo flujo por feto que en camadas de menor tamaño, repercutiendo en su crecimiento (Pere and Etienne 2000; Wu *et al.* 2008).

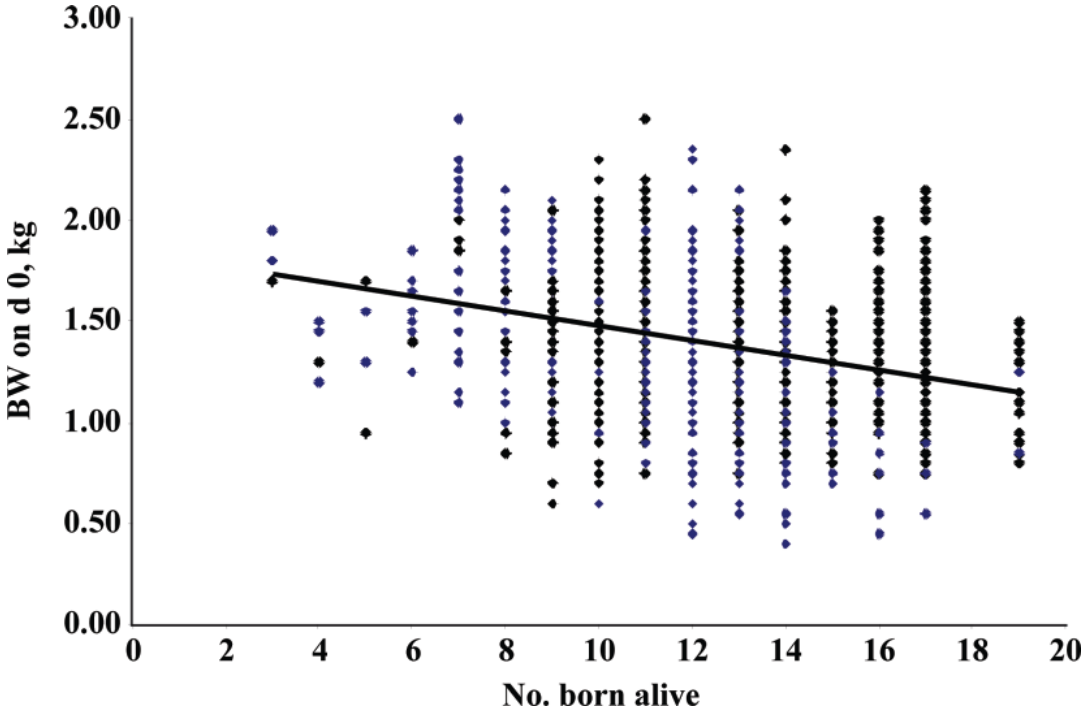


**Figura 2.5.** Regresión del número de nacidos vivos por camada (eje x) y peso al nacimiento (eje y; kg). Ecuación: y = -0.0374x + 1.85, R2= 0.092 (Beaulieu et al. 2010).

## Placenta

La disponibilidad de espacio uterino es clave para el desarrollo de la placenta. Este órgano, en crecimiento hasta el día 70 de gestación, juega un papel clave en el transporte de nutrientes, gases y residuos entre la circulación materna y fetal (Reynolds *et al.* 2006). De hecho, se ha observado en diferentes estudios que una buena eficiencia placentaria (*peso del lechón/peso de la placenta*) es un factor clave para un buen desarrollo prenatal y un mayor peso (Wu *et al.* 2008). Actualmente, el concepto de eficiencia placentaria por tamaño de placenta es discutido. Esto se debe a que el desarrollo de mecanismos compensatorios mediante el aumento de pliegues placentarios y densidad vascular en el último tercio de gestación que aumenta la eficiencia, siempre dependerán del tamaño de la placenta; no funcionando adecuadamente en las placentas de fetos de menor tamaño (Vallet *et al.* 2009; Vallet *et al.* 2014).

Una de las fuentes de mayor alteración del crecimiento fetal es el insuficiente transporte de nutrientes a través de la placenta debido a una vascularización insuficiente que depende, en gran medida, del desarrollo placentario (Wootton *et al.* 1977; Foxcroft *et*

*al.* 2007). De hecho, la angiogénesis y el flujo de sangre se encuentran reducidas en los fetos afectados por RCIU, a lo que se une un metabolismo celular alterado y con una menor expresión de proteínas relacionadas con transportadores de nutrientes y producción de ATP (Reynolds *et al.* 2006; Chen *et al.* 2015). La alteración del metabolismo y la posible hipoxia, por un suministro inadecuado, incrementa el estrés oxidativo, de por si mayor en estos fetos, y desencadena una inflamación de grado bajo que agrava los efectos de la RCIU (Biri *et al.* 2007; Li *et al.* 2013; Jones *et al.* 2014).

**Alimentación de la cerda durante la gestación**

Otro factor importante es la alimentación de la cerda durante la gestación. La estrategia tradicional era aplicar una restricción alimentaria, para evitar que el engrasamiento durante la gestación tuviera efectos negativos. Como por ejemplo, sobre las ubres disminuyendo la producción de leche o en la eficiencia reproductora de las hembras, en especial en cerdas jóvenes. En el caso de cerdas nulíparas y de primer y segundo parto este aspecto es vital, ya que a las necesidades de gestación se unen las necesidades de crecimiento y una restricción alimentaria puede tener consecuencias nefastas en el ciclo reproductivo presente y posterior. Además, este tipo de práctica puede producir una inadecuada provisión de aminoácidos para la gestación y un crecimiento subóptimo de la placenta y de los fetos (Chen *et al.* 2015). Esto tendrá especialmente un mayor efecto negativo en el PN durante el tercer tercio de gestación que durante el primer o segundo tercio, ya que es cuando se produce el crecimiento exponencial de los fetos (Kitchen and Pérez 2003; McPherson *et al.* 2004; Wu *et al.* 2006; Noblet *et al.* 2007). En los casos de restricción, la cerda entra en una fase catabólica en respuesta al balance negativo de energía antes de lo esperado, lo que generara una resistencia a la insulina prolongada que disminuye la síntesis de óxido nítrico (NO) y, por ello, el suministro placentario, al ser el principal regulador del flujo sanguíneo uteroplacentario (Wu *et al.* 2010). En la cerda ibérica, por temor a un engrasamiento excesivo tradicionalmente se ha restringido el consumo en diferentes fases de la gestación para evitar problemas posteriores (Buxadé Carbó and Daza Andrada 2001). En estudios realizados se han observado reducciones de PN medio de la camada realizando restricciones del 50% durante los dos últimos tercios de gestación en cerdas ibéricas (Gonzalez-Bulnes *et al.* 2012a; Ovilo *et al.* 2014c).

Cuando la restricción es muy severa durante toda la gestación el PN decrece y presenta mayor variabilidad y los pesos de algunos órganos y el desarrollo óseo y muscular se ven alterados especialmente por la disminución de aporte proteico (Baker *et al.* 1969; Atinmo

*et al.* 1974; Rehfeldt *et al.* 2004). Sin embargo, si la reducción es moderada o son cerdas de baja prolificidad no habrá efecto sobre el PN, gracias a la capacidad de la cerda de movilizar sus reservas (Atinmo *et al.* 1974; Anderson 1975). En un estudio realizado en la raza ibérica, los lechones de cerdas bajo malnutrición por defecto y por exceso durante toda la gestación tuvieron un PN similar a los lechones control, pero mayor que los lechones de madres con restricción sólo en los dos últimos trimestres (Barbero *et al.* 2013). Esto indicaría una respuesta adaptativa de los embriones y de la cerda a la malnutrición materna en parte relacionada con la secreción de insulina y la sensibilidad periférica de los tejidos a esta hormona, presentándose problemas cuando hay inconsistencia entre los periodos pre- y post-implantación (Watkins *et al.* 2008; Gonzalez-Bulnes and Ovilo 2012; Gonzalez-Bulnes *et al.* 2012a; Gonzalez-Bulnes *et al.* 2013b).

### *2.3.3.2. Efectos postnatales de la variabilidad del peso al nacimiento*

La media de PN disminuye al aumentar la prolificidad situándose entre 1.2-1.6 kg, con 1.45 kg como el valor más común en las razas de genética magra seleccionadas, proviniendo los datos en su mayoría del cruce de las razas Landrace y Large White (Quiniou *et al.* 2002; Baxter *et al.* 2008; Jourquin *et al.* 2016). Para identificar a los animales afectados por la RCIU, siempre se ha considerado un BPN respecto a la distribución normal de PN como la clave, quedando el punto de corte en la mayoría de los estudios entre 1-1.22 kg (Quiniou *et al.* 2002; Baxter *et al.* 2008; Blomberg *et al.* 2010; Wang *et al.* 2017). Los lechones de BPN, presentan una mayor mortalidad en las primeras semanas de vida, siendo el periodo de mayor mortalidad en la producción porcina tras el parto (Milligan *et al.* 2002; Quiniou *et al.* 2002). Estudios recientes destacan el PN de 1.11-1.13 kg como el punto de corte de mayor mortalidad durante la lactación, siendo superior entre 0.5-1 kg de PN en lechones de raza magra (Feldpausch et al. 2016; Jourquin et al. 2016), incluso tomando la misma cantidad de calostro por kg de PN que animales de mayor PN (Jourquin and Morales 2018). Esta menor supervivencia se ve afectada por el BPN en sí, ya que puede condicionar su viabilidad respecto a lechones de PN normal (PNN) y por la competitividad dentro de camada. La falta de homogeneidad en los PN genera una competencia desigual durante la lactación con sus hermanos de mayor PN y disminuye, mejorando el desarrollo de los lechones de BPN, cuando el PN es más homogéneo (English 1998; Douglas *et al.* 2014), La variabilidad de PN aumenta

en las camadas de mayor tamaño al aumentar los lechones de BPN (van der Lende and de Jager 1991; Milligan *et al.* 2002; Quiniou *et al.* 2002).

Al nacimiento, los lechones de BPN presentan diferencias en el tamaño y composición corporal, en general, y en diferentes órganos y estructuras en particular. Estos lechones tienen un menor porcentaje de masa muscular y menor número de miofibrillas, mientras que el peso relativo de los órganos al PN es mayor que en los lechones de PN normal (Rehfeldt and Kuhn 2006). También presentan menores reservas de grasa que puede dificultar la termogénesis, crucial en las primeras horas de vida. Se ha observado un mayor peso relativo de órganos vitales para priorizar la supervivencia en los neonatos de BPN, como son el cerebro, el hígado y el intestino, mientras que otros órganos como el corazón o los riñones han tenido un peso relativo menor; también en lechones ibéricos (Wu *et al.* 2006; Vuguin 2007; Torres-Rovira *et al.* 2013; Gonzalez-Bulnes *et al.* 2015). Además, a nivel intestinal, los neonatos de BPN presentan menor densidad de vellosidades y alteración en la colonización bacteriana que podría contribuir a una mayor morbilidad a largo plazo (D'Inca et al. 2011).

Durante el crecimiento postnatal, los cerdos de BPN presentan menores PV y GMDP y mayores IC es decir, un peor desarrollo (**Tabla 2.4.**). En parte, está relacionado con el menor número de miofibrillas, generando mayores porcentajes de fibras anormalmente grandes y limitando su potencial de crecimiento muscular mientras se aumenta el crecimiento graso (Rehfeldt *et al.* 2000; Rehfeldt and Kuhn 2006). Entre los cerdos de NPN no hubo muchas diferencias, al diferenciarlos en dos categorías de PN: PN mediano (PNM) y alto PN (APN)

**Tabla 2.4.** Efectos del bajo peso al nacimiento (BPN; kg) en el crecimiento postnatal.

| BPN | Efecto sexo | Efectos[1] | Detalles | Referencia |
|---|---|---|---|---|
| Md≈1 | n/s | ↓ PV destete | | (Milligan *et al.* 2002) |
| Md: 1.27 \| no < 1 | n/s | ↓ crecimiento en lactación<br>= resto del crecimiento | PV y GMDP<br>PV y GMDP | (Bee 2004) |
| < 1 | n/s | ↓ crecimiento en lactación y transición<br>↑ Edad a matadero | PV y GMDP | (Quiniou *et al.* 2002) |
| 0.8-1.1 | n/s | ↓ crecimiento en lactación y transición<br>↓ GMDP Total | PV y GMDP | (Gondret *et al.* 2005) |

| BPN | Efecto sexo | Efectos[1] | Detalles | Referencia |
|---|---|---|---|---|
| | | ↑ Edad a matadero | | |
| 0.75-1.25 | Sólo ♀ | ↓ crecimiento<br>↑ Edad a matadero | ↓ PV y GMDP<br>↑IC Cebo | (Gondret *et al.* 2006) |
| <1.20 \| no <0.8 | n/s | ↓ crecimiento | PV y GMDP | (Rehfeldt and Kuhn 2006) |
| Md: 1.41 | Sólo ♂ | ↓ crecimiento desde cebo<br>↑ Edad a matadero | ↓ GMDP ↑IC desde destete | (Bérard *et al.* 2008) |
| <1.22 \| no <0.8 | Si. Sin Interacciones | ↓ crecimiento | PV y GMDP | (Rehfeldt *et al.* 2008) |
| 0.8-1.2 | n/s | ↓ crecimiento<br>↑ Edad a matadero | PV y GMDP | (Beaulieu *et al.* 2010) |
| <1.20 \| no <0.8 | ♀<br>↓ crecimiento en cebo | ↓ crecimiento en lactación y transición<br>↑ Edad a matadero | PV y GMDP | (Bérard *et al.* 2010) |
| 0.8-1.2 | n/s | ↓ crecimiento menos en el acabado | PV y GMDP | (Alvarenga *et al.* 2013) |
| Md 1.26 -1.4 | ♀<br>↓ crecimiento en cebo. Interacciones. | ↓ crecimiento en lactación | PV y GMDP | (Pardo *et al.* 2013) |
| Md: 1.12 | ♀ BPN ↓GMDP Total | ↓ crecimiento<br>↑ Edad a matadero | PV y GMDP | (Smit *et al.* 2013) |

[1] ↑ mayor; ↓ menor

PV: Peso vivo; GMDP: Ganancia media diaria de peso; IC: Índice de conversión

Md: Media; DG: Días de gestación; PN: Peso al nacimiento; n/s: sin indicar; ♂: Macho; ♀: Hembra

A nivel de la canal y la calidad de carne también se observan parámetros alterados en los cerdos de BPN que indican una menor calidad; aunque no en todos los estudios, lo que puede estar relacionado con rangos de BPN más elevados o tamaños de muestras muy bajos (**Tabla 2.5.**). Parte de los parámetros de baja calidad se relacionan con un mayor porcentaje de miofibrillas gigantes (Fiedler *et al.* 2004; Gondret *et al.* 2006). Además, se ha observado una distribución alterada del receptor de la leptina a nivel hipotalámico que

podría estar relacionado con el control del apetito y su mayor cantidad de grasa (Attig *et al.* 2008).

**Tabla 2.5.** Efectos del bajo peso al nacimiento (BPN; kg) en la canal y la calidad de carne.

| BPN | Efecto sexo | Efectos[1] | Detalles | Referencia |
|---|---|---|---|---|
| Md: 1.27 \| no < 1 | n/s | ↑% tejido adiposo | | (Bee 2004) |
| 0.8-1.1 | n/s | = composición de canal<br>≠ Miofibrillas<br>= GIM =pH | = tejido adiposo<br>↑Grosor ↓ nº | (Gondret *et al.* 2005) |
| 0.75-1.25 | Sólo ♀ | ↑ Grasa ↑ GIM<br>↓ Rendimiento de piezas<br>≠ Miofibrillas↓Terneza | Subcutánea y Visceral<br>Jamón y LD<br>↑Grosor ↓ nº | (Gondret *et al.* 2006) |
| <1.20 \| no <0.8 | n/s | ↓ Canal ↑ Grasa Visceral<br>↑ Perdidas por goteo<br>≠ Miofibrillas | ↑Grosor ↓ nº | (Rehfeldt and Kuhn 2006) |
| Md: 1.41 | Sólo ♂ | ↑ Rendimiento de la canal<br>= composición de canal<br>≠ Color | = % tejido adiposo | (Bérard *et al.* 2008) |
| <1.22 \| no <0.8 | ♀ BPN ↓% magro<br>↑ Grasa | ↓ Canal ↑ Grasa Visceral<br>↓ Área Muscular ↑GIM | | (Rehfeldt *et al.* 2008) |
| 0.8-1.2 | n/s | = composición y rendimientos de canal<br>↑ GIM ↓ MS muscular<br>≠ Miofibrillas | ↑oxidativo ↓ nº | (Beaulieu *et al.* 2010) |
| <1.20 \| no <0.8 | ♀ ↓ grasa subcutánea<br>↑Rendimiento magro | ↑ Grasa<br>↓ Rendimiento de piezas | Subcutánea y Visceral<br>Jamón | (Bérard *et al.* 2010) |
| 0.8-1.2 | n/s | ↓Canal ↓Rendimiento de piezas<br>≠ Miofibrillas | ↓ nº | (Alvarenga *et al.* 2013) |
| Md 1.26 -1.4 | ♀ ↓% grasa<br>↑Rendimiento | = | | (Pardo *et al.* 2013) |
| 0.8-1.2 | Sólo ♂ | ↓ Canal<br>↑Dureza =AG | | (Alvarenga *et al.* 2014) |
| <1.3 | n/s | ↑ GIM ≠ AG | ↑C16:1 ↓20:5n-3 | (Rekiel *et al.* 2014) |

| BPN | Efecto sexo | Efectos[1] | Detalles | Referencia |
|---|---|---|---|---|
| | | ≠ Color ↑Dureza | | |

[1] ↑ mayor; ↓ menor; = sin efecto, ≠ diferencias
LD: *Longissimus dorsi*; MS: Materia Seca; AG: Ácido Graso;
Md: Media; PN: Peso al nacimiento; n/s: sin indicar; ♂: Macho; ♀: Hembra

A lo largo de la fase postnatal el BPN tiene repercusión en la morbilidad, en el crecimiento y en la calidad de la canal y de la carne, como hemos podido ver en las tablas anteriores (Wu *et al.* 2006). Estos efectos están condicionados por la aparición de fenómenos de *programación* prenatal que mediante cambios epigenéticos producen alteraciones metabólicas (Ji *et al.* 2017). Estos cambios son modificaciones heredables en la función de los genes que no afectan la secuencia del ADN y se pueden producir a través de diferentes mecanismos; la metilación de ADN, cambios en histonas y cromatina y la actuación de ARN no codificante (Goldberg *et al.* 2007). Estas modificaciones se han relacionado con los cambios fenotípicos observados en la descendencia de hembras gestantes que han sufrido escasez de nutrientes durante la gestación (Heijmans *et al.* 2008; Godfrey *et al.* 2011; Broholm *et al.* 2016). Los cambios epigenéticos se producen para adaptar la función del genoma a las condiciones ambientales y así aumentar las probabilidades de supervivencia y mejorar el desarrollo en el periodo postnatal en un ambiente exterior hostil. Sin embargo, si a nivel prenatal se predice un ambiente postnatal de restricción, el animal tendrá un "*epigenotipo ahorrador*" que en un ambiente con exceso de nutrientes hará que su metabolismo sea más eficiente a la hora de almacenar nutrientes. Por otro lado, parte de los cambios epigenéticos inducidos por la *programación* prenatal pueden producir modificaciones a nivel multigeneracional afectando a la descendencia (Ji *et al.* 2017). En el cerdo ibérico, se han observado alteraciones transgeneracionales como una menor edad de pubertad y prolificidad en cerdas y mayor adiposidad y un perfil lípido alterado en lechones al destete (Gonzalez-Bulnes *et al.* 2013a; Gonzalez-Bulnes *et al.* 2014; Gonzalez-Bulnes *et al.* 2016a).

El sexo de la progenie es también un factor importante a evaluar puesto que puede modular los efectos del PN. Al nacimiento, los machos de la raza ibérica tienen mayor peso que las hembras como se ha observado en las razas magras (Eiby *et al.* 2013; Torres-Rovira *et al.* 2013), Sin embargo en caso de malnutrición, las hembras ibéricas presentan un mejor desarrollo prenatal presentando un peso similar al de los machos, un mejor desarrollo de los órganos vitales y una menor expresión de péptidos anorexigénicos en el hipotálamo (Torres-Rovira *et al.* 2013; Ovilo *et al.* 2014c; Gonzalez-Bulnes *et al.* 2015;

Gonzalez-Bulnes *et al.* 2016a). En cerdos de razas magras, durante el periodo postnatal se ha encontrado un peor crecimiento de las hembras durante el periodo de cebo y una mayor edad para alcanzar el peso a matadero que en los machos (Bérard *et al.* 2010; Schinckel *et al.* 2010). Destacan las hembras de BPN por ser el grupo con peor crecimiento; aunque no hay gran cantidad de estudios que valoren esta característica como se puede observar en la **Tabla 2.5**. No hay datos sobre la relación del PN y el sexo en cerdos ibéricos, sólo sobre efectos de la restricción alimentaria durante la gestación. En casos de restricción materna se ha observado que las hembras descendientes presentan un mayor crecimiento que los machos durante la lactación, sin haber diferencias entre los lechones de las madres control. Más tarde, en la fase de crecimiento las hembras de madres restringidas llegaron a superar en PV de las hembras controles, pero los machos de madres restringidas no alcanzaron a sus pares controles (Gonzalez-Bulnes *et al.* 2012a; Barbero *et al.* 2013; Ovilo *et al.* 2014c). Por último, el efecto del sexo en la calidad de la canal y de la carne respecto al PN está poco estudiado como muestra la **Tabla 2.6.**, en la que se pueden ver incluso efectos contradictorios. En el caso del cerdo ibérico, se ha observado una mayor cantidad de grasa visceral en hembras de madres restringidas que en los machos de las mismas madres, sin encontrar diferencias en el grupo control (Barbero *et al.* 2013).

Variabilidad en la producción porcina

La producción cárnica moderna, particularmente en el sistema de producción intensivo, requiere uniformidad en el peso a sacrificio y en la calidad de la canal de los lotes de animales que llegan a matadero (King 1999; Hennessy 2005). Una menor variabilidad permite un mejor manejo a nivel industrial y una mayor satisfacción de los consumidores que exigen una calidad de producto homogénea. Para los productores de cerdo, el interés es económico ya que puede acarrear penalizaciones directas en el matadero si no cumplen con los pesos y la calidad de la canal acordadas con la industria cárnica (Douglas *et al.* 2014; López-Vergé *et al.* 2018). Al mismo tiempo, la falta de homogeneidad reduce las ganancias para los productores al generar un incremento de los días en producción, especialmente en el periodo de cebo que es el más costoso (SIPConsultors 2017), y puede dificultar el manejo sanitario al no realizarse vacíos sanitarios en los tiempos adecuados (**Figura 2.6.**).

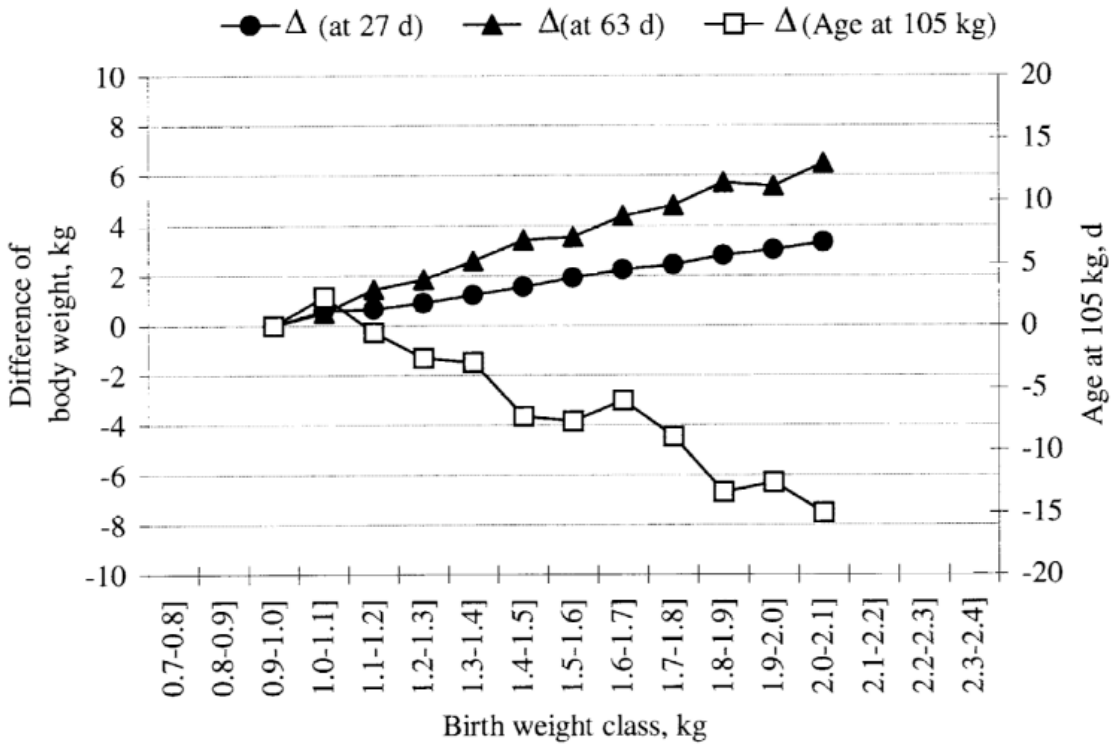


**Figura 2.6.** Diferencias en el peso vivo (PV, kg; eje y izquierda) al destete (27 días, ●) y al principio del cebo (63 días; ▲) y en la edad al sacrificio con un PV estandarizado de 105 kg (□; eje y derecha), cuando el valor de 1 kg PN (kg; eje x) es usado como referencia (Quiniou et al. 2002).

En el cerdo ibérico, hay poca información, pero se sabe que la homogeneidad es menor que en otras razas, los lechones ibéricos muestran al destete un diez por ciento más de PV extremos que lechones de razas magras (Arévalo Mozos and Palomo Yagüe 2008; Soto et al. 2010). Estas diferencias aumentarían con la edad (López-Vergé *et al.* 2018), especialmente importante en la raza ibérica por los largos periodos productivos; por ello, frecuentemente la edad entre lechones de la misma camada en alcanzar el peso objetivo a matadero puede variar entre 3 o 4 semanas (Olivares Moreno 2009; Ayuso Hernando 2016). La optimización de los sistemas de producción de la raza ibérica para la homogeneidad en los lotes de producción requiere el estudio de las causas de variación del peso al sacrificio, el crecimiento, la composición de la canal y la calidad de carne en esta raza. En estudios realizados en cerdos de razas magras se ha considerado, la variación del PN y el sexo como factores que pueden afectar a la homogeneidad final (**Figura 2.7.**; Le Dividich 1999; Klindt 2003; Schinckel *et al.* 2010; Egea *et al.* 2016; López-Vergé *et al.* 2018).

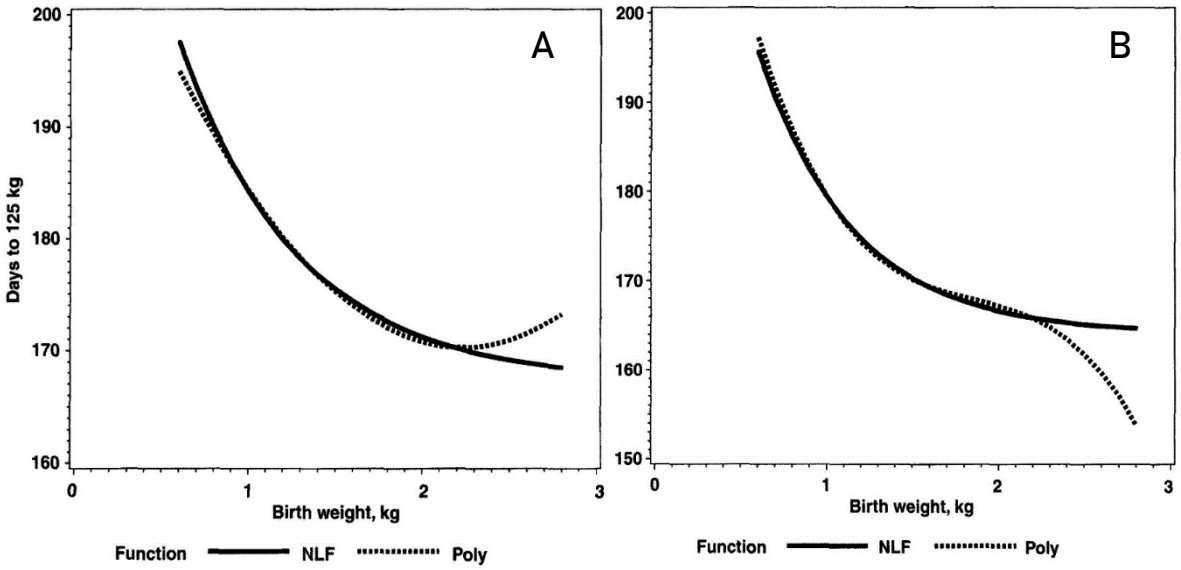


**Figura 2.7.** Relación del peso al nacimiento (eje x; kg) y los días utilizados en alcanzar los 125 días (eje y) mediante funciones polinomiales (poly) y no lineares (NLF) en hembras (A) y machos (B; Schinckel et al. 2010)

## 2.4. Estrategias para homogeneizar el peso al nacimiento

Como se ha descrito anteriormente, el PN está fuertemente asociado a características importantes para la rentabilidad económica de la producción porcina. Desafortunadamente, el PN tiene una baja heredabilidad por lo que es necesario trabajar con otro tipo de estrategias para aumentarlo y disminuir así sus efectos negativos (Wittenburg et al. 2008). Las principales estrategias son las estrategias nutricionales prenatales destacando la utilización de nutrientes que puedan ayudar a mejorar la condición corporal de las cerdas, el desarrollo placentario y por ende su flujo sanguíneo o a mejorar el estado oxidativo/antioxidante, inflamatorio e inmunomodulador a nivel de placenta y feto (Wu et al. 2010; Mordhorst and Prather 2017). Con estas intervenciones se pretende aumentar el intercambio materno-fetal o prevenir los efectos negativos del estado oxidativo o inflamatorio, en especial en los fetos que sufran RCIU, permitiendo a los fetos crecer más eficientemente y, también, obtener más uniformidad en las camadas. Existen diferentes experimentos sobre el efecto de distintos compuestos nutricionales durante la gestación como son aminoácidos, AG, vitaminas y antioxidantes, en su mayoría usados ya en cerdas (**Tabla 2.6.**).

**Tabla 2.6.** Efectos suplementación maternal durante la gestación en los neonatos.

| Tipo | **Compuesto** | **Animal** | **Efectos** | **Referencias** |
|---|---|---|---|---|
| AA | Arginina | Cerdo | Incrementa el tamaño de camada, los lechones nacidos vivos y el PN. También mejora el estado oxidativo. | (Wu *et al.* 2010; Wu *et al.* 2013) |
| | Glutamina | Cerdo | Aumenta el número de lechones vivos el PN y su uniformidad en la camada. Si se mescla con Arginina también se producen estos efectos. | (Wu *et al.* 2011) |
| | Prolina | Cerdo | Incrementa el tamaño de camada, los lechones nacidos vivos y el PN. | (Gonzalez-Añover and Gonzalez-Bulnes 2017) |
| AG | AG poliinsaturados n-3 y n-6 | Cerdo | Mejora el crecimiento postnatal mejorado, disminuye de la mortalidad predestete y el PN, con resultados mixtos sobre el efecto del tamaño de la camada. | (Rooke *et al.* 2001; Spencer *et al.* 2004; Webel *et al.* 2004) |
| Vitamina | Ácido fólico (B9) | Cerdo | Se aumenta el metabolismo hepático en lechones afectados por IUGR normalizando su expresión génica y función mitocondrial. | (Liu *et al.* 2011; Liu *et al.* 2012) |

| Tipo | Compuesto | Animal | Efectos | Referencias |
|---|---|---|---|---|
| | Retinol (A) | Cerdo | Reducir el retinol durante el primer trimestre ayuda a tener mayor uniformidad de PN | (Antipatis *et al.* 2008) |
| Polifenol | Quercetina (Flavonoide) | Roedor | Restauración del desarrollo fetal y prevención de hiperglucemia postnatal, resistencia a la insulina, obesidad, hipertensión y osteoporosis temprana. Acelera el desarrollo reproductivo en hembras y puede afectar a la implantación | (Johnson *et al.* 2009; Wu *et al.* 2014; Cao *et al.* 2016; Santangelo *et al.* 2016) |
| | Isoflavonas (Flavonoide) | Roedor | Decrece el estrés oxidativo y disminuye la hipertensión en la progenie. Sin embargo, se han visto problemas con la diferenciación sexual y por acelerar el desarrollo reproductivo en hembras. También induce cambios epigenéticos. | (Bonacasa *et al.* 2011; Jefferson and Williams 2011; Chango and Pogribny 2015) |
| | Resveratrol | Roedor | Mejora el estado oxidativo y apoptosis asociada con embriopatía diabética y previene retrasos en el desarrollo embrionario. Además, mejora el estado oxidativo y la vascularización del riñón en casos de RCIU. | (Singh *et al.* 2011; Costa *et al.* 2016) |

AA: Aminoácido; AG: Ácido Graso; PN: Peso al nacimiento; RCIU: Restricción de crecimiento intrauterino.

Entre las posibles estrategias nutricionales se encuentra la suplementación con polifenoles. Estos compuestos están presentes en multitud de plantas y sus productos derivados, puesto que son productos secundarios de su metabolismo. Entre los más conocidos están el ácido gálico, la genisteína, el resveratrol y la curcumina. Los polifenoles son conocidos por ser potentes antioxidantes con una acción doble, puesto que capturan e inhiben especies reactivas de oxígeno y, al mismo tiempo, aumentan la capacidad antioxidante del plasma (Ly et al. 2015). También presentan actividad antiinflamatoria, antimicrobiana, antiviral y antitumoral. Es principalmente gracias a su actividad antioxidante y antiinflamatoria que los polifenoles producen efectos positivos durante la gestación (T**abla 2.6.**). De hecho, el tratamiento con un fármaco con gran poder antioxidante, alopurinol, ha mostrado ser potencialmente útil para reducir los efectos de la RCIU sin efectos adversos (Prickaerts et al. 2014). Aunque el uso de polifenoles durante la gestación ha demostrado tener efectos beneficiosos para la RCIU, también se han encontrado algunos posible riesgos como son la posibilidad de interferir en la implantación, la aceleración del desarrollo reproductivo en hembras de la descendencia,

la alteración de genes para el desarrollo de la reproducción en la descendencia o la inducción de cambios epigenéticos por modificaciones en las histonas o cambios en el microARN (T**abla 2.6.**).

Entre las plantas ricas en polifenoles que tenemos en España se encuentra el olivo. En este árbol se pueden encontrar diferentes compuestos fenólicos en la aceituna (oliva) o en sus hojas y, por ello, en el aceite de oliva o sus residuos de producción. Debido a que España es el país con mayor cantidad de hectáreas dedicadas al cultivo de olivares y el primer productor de aceite de oliva a nivel mundial, favorecer el uso de sus polifenoles puede ser beneficioso (Eurostat). La cantidad de polifenoles en los productos derivados del olivo varía, entre 150-650 mg/kg en el aceite y 1.5-8.5 g/ kg de olivas, aunque siempre habrá mayor cantidad en las hojas que en los frutos, y depende de la variedad del árbol, el ambiente del olivo y el procesamiento de los derivados (Delgado-Pertíñez *et al.* 2000; Ranalli *et al.* 2009; Visioli and Bernardini 2011). Los polifenoles derivados del olivo son una fracción compleja constituida por más de 30 compuestos diferentes entre los que destacan la oleuropeína, el tirosol y el hidroxitirosol (HT). La oleuropeína que es polifenol con mayor presencia, seguido por el HT, y es en muchas ocasiones el polifenol utilizado para valorar la cantidad de polifenoles en los productos como en el caso de las hojas donde representa entre 1-14 mg/g de hoja frente a las olivas que sitúa por debajo de 1 mg/g de oliva (Fabbri *et al.* 2008; Vogel *et al.* 2014).

El aceite de oliva y otros derivados del olivo cada vez son más usados en experimentos para estudiar los posibles efectos beneficiosos de sus polifenoles sobre los seres vivos. Aunque mayoritariamente los estudios sobre sus propiedades se realizan en roedores, en el cerdo se ha podido observar que el uso de extractos de aceite de oliva a dosis de 500 mg/kg de pienso suprime los efectos de la inflamación crónica subclínica a nivel intestinal mejorando la integridad intestinal sin afectar a la microbiota (Liehr *et al.* 2017). También se han usado en cerdos y en otras especies ganaderas productos derivados del olivo como suplemento para mejorar la calidad de la carne usando dosis entre 50-100 g/kg de pienso para el aceite de oliva u hojas de olivo y dosis entre 1.5-3 mg/kg de pienso de extracto de aceituna o alperujo con resultados positivos en algunas características como el color, las perdidas por goteo o el estatus oxidativo (Nuernberg *et al.* 2005; Paiva-Martins *et al.* 2009; Apeleo Zubiri 2017). Además, se ha detectado menor expresión de genes relacionados con la síntesis de prostanoides en cerdos alimentados con dietas ricas en aceite de oliva alto oleico en comparación con dietas ricas en carbohidratos (Ovilo *et al.* 2014a). Respecto a su uso como suplemento

durante la gestación, no hay hasta el momento ningún estudio que aporte datos sobre ello. Por otro lado, entre el conjunto de polifenoles del olivo hay dos que se utilizan de manera individual en algunos experimentos y son la oleuropeína y el HT. Este último, que es el polifenol más abundante en los residuos procedentes de la fabricación del aceite de oliva, es especialmente interesante por su menor peso molecular y estructura química que permite un mejor manejo para su uso a nivel industrial (Bitler *et al.* 2005; Visioli and Bernardini 2011).

### 2.4.1. Hidroxitirosol

El HT (3,4-dihidroxifeniletanol; **Figura 2.8.**) es el segundo compuesto fenólico por abundancia, de tipo feniletanoide o alcohol fenólico, presente en la oliva y, en mayor concentración, en las hojas del olivo. Destaca por su importante capacidad antioxidante, incluso superior a la vitamina E, lo que le convierte en un suplemento nutricional de gran interés (Visioli and Bernardini 2011). El HT también actúa como regulador del metabolismo y tiene propiedades antiinflamatorias, inmunomoduladoras y antivirales; además se sabe que inhibe la adipogénesis y que es hipoglucemiante, neuroprotector, antihipertensivo e hipocolesterolémico (Vilaplana-Pérez *et al.* 2014; Vogel *et al.* 2014). Aunque gran parte de los estudios realizados sobre el HT han sido *in vitro*, cada vez se realizan mayor cantidad de experimentos *in vivo*. Como ejemplo, en sus inicios fue especialmente usado en experimentos para inhibir la oxidación de lipoproteínas de baja densidad, confirmándose más tarde un efecto cardioprotector en estudios *in vivo* en animales (Gonzalez-Santiago et al. 2006; Visioli and Bernardini 2011).

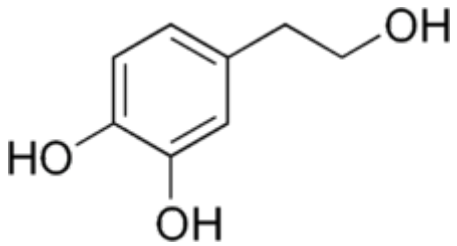


**Figura 2.8.** Estructura química del hidroxitirosol.

Otras propiedades también han sido estudiadas en estudios *in vivo* en animales, en su mayoría roedores, y humanos. En parte de estos estudios se puede encontrar el uso conjunto del HT con la oleuropeína, descartando los que usan el conjunto total de polifenoles del olivo. Como en un estudio dónde disminuyeron los triglicéridos en ratones diabéticos al recibir 8 y 16 mg/kg de PV de un extracto con los dos polifenoles, mostrando también un efecto hipoglucemiante dosis dependiente (Jemai *et al.* 2009). También se ha

visto que el HT puede reducir la formación de proteínas glicadas relacionada con la *diabetes mellitus* (Kontogianni *et al.* 2013). En otro experimento realizado en ratas con dosis de HT entre 2.5 y 10 mg/kg de PV/día, también se encontró un efecto hipocolesterolémico junto con una disminución de la peroxidación lipídica que se relaciona con sus propiedades cardioprotectoras (Fki *et al.* 2007). En relación a estas propiedades, se ha observado la disminución de la producción de tromboxano-2 en pacientes diabéticos tipo 1 usando dosis de HT entre 12.5 y 25 mg/día durante 4 días (Leger *et al.* 2005) y la disminución de lesiones de arterioesclerosis y un efecto antioxidante en conejos con una dosis de 4 mg/kg de PV durante un mes (Gonzalez-Santiago *et al.* 2006). Sobre sus propiedades antiinflamatorias, se ha observado una reducción del TNF-μ en ratones a partir de una dosis de HT de 25.5 mg/kg de PV/día, disminuyendo más de un 90% con una dosis de 85 mg/kg de PV (Bitler *et al.* 2005). También ha demostrado ser efectivo para disminuir el dolor en pacientes con artritis tras administrar dosis de HT de 6.8 mg /día durante 60 días y marcadores inflamatorios en pacientes sanos con una única dosis de 15 mg (Bitler *et al.* 2007; Bogani *et al.* 2007). Aunque en casi todos los estudios el HT muestra propiedades antioxidantes, suelen usarse dosis superiores a 0.1 mg/kg de PV; sin embargo, en un estudio en conejos se logró reducir el estrés oxidativo inducido por el humo del tabaco usando bajas dosis de HT (0.4 μg/kg de PV/día) en un tratamiento de 2 días (Visioli *et al.* 2000). Por otro lado, también se ha observado en conejos un efecto neuroprotector a nivel cerebral utilizando dosis de 100 mg/kg de PV durante 12 días (Schaffer *et al.* 2007).

Respecto a sus características farmacocinética, la absorción del HT como en el resto de fenoles presentes en el aceite de oliva es dosis dependiente y se considera alta y con una vida media en plasma de 2.43h, lo que condiciona su biodisponibilidad al favorecerse su excreción urinaria (Miro-Casas *et al.* 2003). También presenta una alta estabilidad y se considera compuesto seguro, ya que no se han demostrado efectos tóxicos tras su administración aguda y subcrónica, incluso a altas dosis como son 2 g/kg de PV/día (D'Angelo *et al.* 2001; Soni *et al.* 2006; Bulotta *et al.* 2014; Vilaplana-Pérez *et al.* 2014). Su eliminación se realiza a través de la orina en forma de conjugados glucurónidos y otras formas de conjugación también detectadas (Caruso *et al.* 2001; Miro-Casas *et al.* 2003). De hecho, su uso se ha relacionado con un aumento en la eliminación de alcohol homovanílico y el ácido homovanílico que son el resultado de la metilación de la molécula de HT sin oxidar y oxidada, respectivamente (Caruso *et al.* 2001). Se sabe que tras su ingesta se asocia a lipoproteínas decreciendo la oxidación de las mismas (Gonzalez-Santiago *et al.* 2010). Sin embargo, se deben realizar más estudios ya que se ha

encontrado que el metabolismo de esta molécula depende de la especie en cuestión y de su matriz de administración (Visioli *et al.* 2003).

Los mecanismos de acción del HT y otros compuestos fenólicos del aceite de oliva continúan siendo investigados. Actualmente, se relaciona al HT con la activación de las sirtuinas (Sirt), que son desacetilasas NAD-dependientes, que a su vez activan el factor de transcripción Nrf2 y con ello sus genes dependientes estimulando la producción de enzimas de la Fase II (anti-xenobióticas) y antioxidantes lo que aumenta la síntesis de glutatión y las rutas de detoxificación (Corona *et al.* 2007; Zhu *et al.* 2010; Visioli and Bernardini 2011; Bayram *et al.* 2012; Rigacci and Stefani 2016). Además, algunos estudios indican una posible interferencia en la proliferación celular. También se sabe, principalmente por experimentos *in vitro*, que el HT inhibe la agregación plaquetaria y las enzimas proinflamatorias y estimula la óxido nítrico sintetasa en su forma inducible, pero no en su forma endotelial (Petroni *et al.* 1995; Petroni *et al.* 1997; Visioli *et al.* 1998; de la Puerta *et al.* 1999; Schmitt *et al.* 2007).

En consecuencia, debido a las propiedades demostradas en los análisis *in vitro*, principalmente, y a sus características químicas, que facilitan su síntesis, y farmacocinéticas ha habido un aumento de interés en su uso a nivel nutraceútico; especialmente por su relevancia frente a patologías como las enfermedades cardiovasculares, metabólicas y neurodegenerativas y el cáncer (Rigacci and Stefani 2016). Aunque hay diversos estudios sobre los posibles usos y los efectos de HT en los seres vivos es necesario profundizar en estos conocimientos y aumentar las áreas de estudio. Por ejemplo, el HT ha mostrado actuar frente al daño celular, pero hay información limitada sobre los efectos epigenéticos de los polifenoles presentes en la oliva (Visioli and Bernardini 2011). Sin embargo, las similitudes que tienen de actuación con otros polifenoles vegetales a nivel molecular parecen indicar que podrían ser útiles para actuar sobre los cambios epigenéticos que se producen en el genoma (Chango and Pogribny 2015). Tampoco, se han encontrado estudios sobre el efecto del HT a nivel reproductivo ni en relación con el PN, aunque por sus propiedades antioxidantes y antiinflamatorias podría ser de gran interés para disminuir los efectos negativos de la RCIU.

# 3. Planteamiento experimental

Para el desarrollo de esta tesis doctoral se plantearon tres protocolos experimentales. Los experimentos 1 (*Apartado 4.1.*) y 2 (*Apartado 4.2.*) fueron realizados en condiciones de granja comercial. El tercer experimento se realizó en condiciones de granja experimental (*Apartado 4.3.*).

El primer experimento tenía como objetivo proporcionar información, en la raza ibérica, sobre la incidencia de lechones de BPN, la variabilidad del PN dentro de la camada y sus efectos postnatales sobre el crecimiento y la calidad de la canal y la carne. El segundo experimento se orientó hacia el estudio de los efectos postnatales sobre la progenie de una restricción materna alimentaria ligera durante la gestación en las mismas condiciones que el primer experimento. El último experimento tuvo como objetivo evaluar del uso del HT, un polifenol del olivo, como estrategia nutricional para disminuir los efectos negativos de la RCIU en el cerdo ibérico.

## 3.1. Diseño del experimento 1

El experimento 1 fue realizado en una granja comercial perteneciente a la empresa Ibéricos de Arauzo S.L. (Salamanca, España) y se utilizaron cerdos de cruce final de ibérico y Duroc al 50%. La granja de madres constaba de 2000 reproductoras y posteriormente los animales del experimento se trasladaron a cebaderos de la misma empresa. Los cerdos que formaron parte del experimento se sacrificaron, una vez alcanzaron el peso a sacrificio (Real Decreto 4/2014), en mataderos comerciales de la provincia de Salamanca.

Para el experimento se utilizaron 406 lechones nacidos vivos de 47 cerdas ibéricas retintas de tercer y cuarto parto. Al nacer los lechones fueron identificados, medidos y pesados. Se calcularon el PN medio y su desviación estándar (DE), así como su coeficiente de variación (CV) según el tamaño de camada. Este tamaño de camada fue dividido, por prolificidad, en 3 grupos (3-6, 7-9 y 10-13 lechones nacidos totales), para estudiar la variabilidad del PN.

Para el estudio de los efectos del PN se realizaron 4 grupos de lechones en función del PN: muy bajo PN (MBPN; ≤0.99 Kg) , bajo PN (BPN; 1.00-1.19 Kg), PN mediano (PNM; 1.20-1.5 Kg) y alto PN (APN; ≥1.54 kg).. El punto de corte para el grupo de menor PN se calculó restando a la media del PN del primer experimento su DE (1.319 ± 0.313 kg). La categoría de BPN incluyó los lechones cuyo PN estaba dentro del percentil 30 (excluyendo los

lechones de MBPN) y la categoría de APN incluyo los lechones cuyo PN estaba a partir del percentil 75.

Al destete (24 días de vida) también se pesaron y midieron, además de medir el grosor de la grasa subcutánea dorsal de 359 lechones. Seguidamente, 240 lechones fueron distribuidos en corrales de 12 animales por sexo y por el PN, de menor a mayor PN, manteniendo esa distribución hasta matadero. Durante este periodo, se registraron los PV de los cerdos a los 71, 110, 150, 180, 215 y 240 días de vida y el día de salida a matadero. Las GMDP fueron calculadas entre esas fechas, para la fase de lactación y transición y para la vida completa de cada cerdo. Además, los consumos por corral fueron recogidos durante la fase de cebo, desde el día 72 hasta el 240 y se calculó el IC entre las fechas de registro de los PV. Así mismo, se registró el grosor de la grasa subcutánea dorsal, diferenciando entre capa interna y externa, a los 110 y 215 días de vida.

En el matadero se tomaron muestras de sangre, de hígado, de *Longissimus dorsi* (LD) y de grasa subcutánea dorsal y se registró el peso y la longitud de la canal y el grosor de la grasa subcutánea dorsal total de 232 cerdos. En las muestras de sangre se analizaron los índices de metabolismo lipídico (colesterol total, triglicéridos y lipoproteínas de baja y alta densidad que transportan el colesterol) y de metabolismo glicémico (insulina, glucosa y fructosamina). Posteriormente, se realizó el análisis de la grasa en los tejidos muestreados y se obtuvo su perfil de AG. En el caso de la grasa subcutánea se analizaron los AG totales y en los otros dos tejidos los AG de la FLN y de la FLP por separado. Posteriormente, se calcularon los totales de los AG saturados, AG monoinsaturados, AG poliinsaturados y los AG n-3 y n-6, así como los índices de insaturación y de desaturación y el ratio de los AG n-6/n-3. Por último, para el LD también se realizó un análisis de pérdidas de agua por goteo.

## 3.2. Diseño del experimento 2

El experimento 2 se realizó en la misma granja que el experimento 1 y con las mismas condiciones de producción, por ello gran parte de los parámetros recogidos en este experimento están ya descritos en el apartado anterior.

Para este estudio, 80 cerdas ibéricas fueron divididas en un grupo control y un grupo restringido (**Figura 3.1.**), Las cerdas del grupo restringido fueron alimentadas con un 70% de sus requerimientos diarios entre el día 30 y 90 de gestación. Al nacimiento, un total de 671 lechones nacidos vivos fueron clasificados según su PN en dos categorías:

lechones de bajo PN (BPN; ≤0.99 Kg) y de PN normal (PNN; ≥1 kg). El punto de corte usado para el grupo de BPN fue el calculado en el primer experimento, descrito en el apartado anterior.

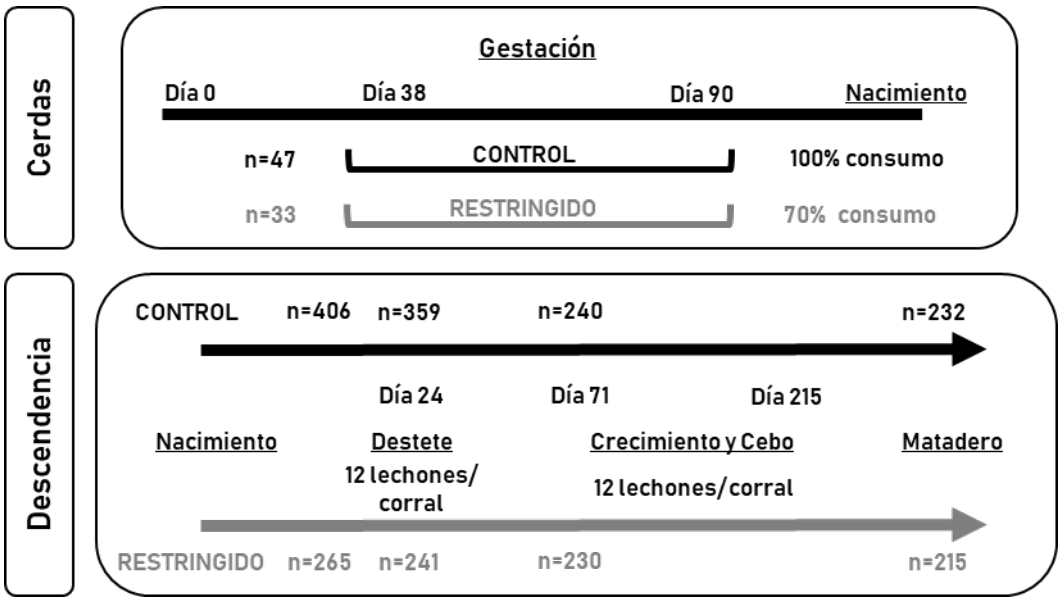


**Figura 3.1.** Diseño del experimento 2.

Al destete, además de los parámetros ya indicados en el apartado anterior se midió el grosor del LD en 600 lechones. Seguidamente, 470 lechones fueron distribuidos en corrales de 12 animales por el tratamiento materno, el sexo y por el PN, de menor a mayor PN hasta matadero. Durante este periodo, se registraron los PV de los cerdos a los 110, 150, 180 y 215 días de vida y el día de salida a matadero. Las GMDP, también, fueron calculadas entre esas fechas, para la fase de lactación y transición y para la vida completa de cada cerdo. Además, los consumos por corral fueron recogidos durante la fase de cebo, entre el día 72 hasta el 215 y se calculó el IC entre las fechas de registro de los PV. Así mismo, se tomaron muestras de sangre y se registró el grosor de la grasa subcutánea dorsal, diferenciando entre capa interna y externa, y del LD a los 215 días de vida. En las muestras de sangre de este experimento se analizaron los mismos índices de metabolismo lipídico y glicémico que en el apartado anterior, salvo la insulina.

En el matadero se tomaron muestras de sangre, de hígado, de LD y de grasa subcutánea dorsal, se registró el peso y la longitud de la canal y el grosor de la grasa subcutánea dorsal de 447 cerdos. Posteriormente, se realizó el análisis de la grasa en los tejidos muestreados y se obtuvo su perfil de AG como en el apartado anterior.

## 3.2. Diseño del experimento 3

El experimento 3 se realizó en las instalaciones de la Granja Animalario del Departamento de Reproducción Animal del INIA (Madrid, España) utilizando cerdos ibéricos puros. Para ello, 20 cerdas ibéricas gestantes fueron divididas en un grupo control (C) y un grupo tratado con hidroxitirosol (HT). Todas las cerdas gestantes fueron restringidas al 50%, para incrementar la incidencia de RCIU y, por tanto, de lechones de BPN. En el caso del grupo HT, la suplementación se aplicó con una dosis de 1.5 mg/día de HT desde el día 35 de gestación hasta el parto. Al nacimiento los lechones fueron identificados y pesados para ser clasificados por el PN en dos categorías de BPN y de PNN, teniendo en cuenta la media y la DE de los lechones del grupo C. En los días 15 y 25 (destete) de vida se registró el PV y se calculó la GMDP para los periodos intermedios entre el nacimiento y el destete. En el destete los cerdos fueron sacrificados, se tomaron muestras de sangre para analizar su estatus metabólico y se registraron el grosor de la grasa subcutánea y del LD y los pesos de diferentes componentes corporales. Entre ellos se encuentran la cabeza, la canal y las vísceras y dentro de estas últimas están el cerebro, el corazón, los pulmones, el hígado, el bazo, los riñones, el páncreas, las glándulas adrenales y el intestino. Con los pesos de las vísceras se calcularon ratios respecto al peso de vísceras totales y también el ratio de la cabeza respecto a la canal. Todos los parámetros fueron estudiados también en base al tamaño de la camada.

# 4. Resultados

# 4.1. Experimento 1

## Piglet birth-weight and sex affect growth performance and fatty acid composition in fatty pigs

Short title: Postnatal effects of birth-weight on fatty pigs

Marta Vázquez-Gómez, Consolación García-Contreras, Susana Astiz, Laura Torres-Rovira, Eugenio Fernández-Moya, Álvaro Olivares, Argimiro Daza, Cristina Óvilo, Antonio González-Bulnes and Beatriz Isabel

Animal Production Science. En evaluación.

Consultar el material suplementario correspondiente en el Anexo 2

# Piglet birth-weight and sex affect growth performance and fatty acid composition in fatty pigs

Short title: Postnatal effects of birth-weight on fatty pigs

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**Abstract.** This study aimed to determine, for fatty breeds, the effects of piglet birth-weight (BIW) and sex and within-litter BIW variation on postnatal growth traits and meat quality. A total of 406 crossbred piglets (half each sex) born to Iberian sows were studied during their postnatal development until slaughter. After birth, piglets were classified in four BIW categories: very low, low, medium and high BIW (VLBIW, LBIW, MBIW and HBIW).

There was a negative effect of low BIW on growth patterns and fatty acid (FA) composition, but effects of litter size and within-litter BIW variation were not found. The VLBIW piglets showed a catch-up growth with HBIW piglets during early postnatal phase ($P<0.005$), but also showed higher feed conversion rate and lower average daily weight gain throughout the period of study ($P<0.05$ for both). Whereas the BIW affected the development during the whole productive life, the sex effect became more important with age. As a result, the period to reach market-weight was 43 and 15 days longer in VLBIW females and males, respectively, than in their HBIW counterparts. BIW and sex also influenced intramuscular fat amount, n-3 FA content and monounsaturated FA composition.

The present study indicates that BIW, modulated by sex, is a critical point for productive traits in fatty pigs. These results set the basis for future strategies to enhance productive efficiency and meat quality of traditional swine breeds.

**Keywords:** Carcass traits, Iberian pig, Lipids, Meat quality, Postnatal development, IUGR.

## Introduction

The increasing demand for high-quality dry-cured products from fatty breeds has changed management practices into more intensive rearing as in lean breeds. The current reproductive strategies for swine production aim to increase prolificacy. However, there is abundant evidence in lean breeds that greater litter size (**LS**) generates a higher within-litter birth-weight (**BIW**) variation (Milligan *et al.* 2002) and a higher incidence of low and very low BIW piglets (**LBIW** and **VLBIW**, respectively; (Foxcroft *et al.* 2006). In brief, the available uterine space is decreased by a high prolificacy and leads to intrauterine growth restriction (**IUGR**) processes, which may cause lower BIW piglets (Wu *et al.* 2006). In fatty pigs, this process may be more severe due to lower prolificacy and uterine capacity of sows.

Lower BIW is associated with to high morbidity, different developmental patterns and changes in body composition and homeostasis, due to prenatal programming through epigenetic changes (Gondret *et al.* 2005b; Gonzalez-Bulnes *et al.* 2016; Ji *et al.* 2017). The result of within-litter BIW variation (**BIWV**) is a high variability in carcass and meat quality in the same feedlot affecting profitability and dry-cured product production (Andretta *et al.* 2016). Moreover, sex contributes to reducing the feedlot homogeneity (Egea *et al.* 2016). Similar impaired postnatal growth patterns have been described for fatty breeds under experimental management (Gonzalez-Bulnes *et al.* 2012b; Barbero *et al.* 2013; Gonzalez-Bulnes *et al.* 2014). However, there is a paucity of data under farm conditions, in spite of the high economic value of these breeds and the main differences with lean breeds (Nieto *et al.* 2012). Fatty pig breeds, such as the Iberian breed, have well-recognized high-quality dry-cured meat products (Lopez-Bote 1998). Thus, the homogeneity of growth patterns and meat quality within feedlot are main goals in fatty pig production because of a lower weight homogeneity than lean breeds (Arévalo Mozos and Palomo Yagüe 2008; Soto *et al.* 2010).

Hence, the present study aimed to determine, for fatty pigs under farm conditions, the effects of BIW, within-litter BIWV and sex on postnatal development and carcass and meat quality at slaughter, including the fatty acid (**FA**) profile.

## Materials and Methods

*Animals and diets*

The study was performed according to the Spanish Policy for Animal Protection RD53/2013, which meets the European Union Directive about the protection of animals used for research. The experiment was specifically assessed and approved (report CEEA 2012/036) by the INIA Committee of Ethics in Animal Research. Animals were housed at a commercial farm, Ibéricos de Arauzo 2004 S.L. (Zorita de la Frontera, Salamanca, Spain).

A total of 406 crossbred piglets (around 50% females and 50% males) born alive after insemination of 47 Iberian sows (Retinto strain) of 3$^{rd}$ and 4$^{th}$ parity with cooled semen from Duroc PIC boars (Genus plc, UK) were involved in this study. Management of sows and offspring was performed in agreement with standard practices in commercial farms, identifing with electronic chips and housing indoors with a controlled temperature. Sows

were allocated in groups until D 101 of pregnancy and then in individual pens until the end of the suckling phase. Newborns were immediately sexed, measured, weighed and identified with earrings after birth. These piglets were allocated with mothers until weaning and then in collective pens. Sows and piglets were fed with standard grain-based diets specific for Iberian pigs (diets are shown in Supplementary Table 1), based on data from De Blas *et al.* (2013).

For experimental purposes, piglets were classified in four BIW categories: VLBIW, LBIW, medium BIW (**MBIW**) and high BIW (**HBIW**). Such classification was performed after considering the mean value of the study group (1.319 ± 0.313 kg; Blomberg *et al.* 2010; Vazquez-Gomez *et al.* 2016). The VLBIW group included piglets with a body weight less than an SD below the mean value (BIW ≤0.99 kg), the LBIW group included piglets at the 30 percentile (after excluding VLBIW piglets; BIW=1.00-1.19 kg), the HBIW group included piglets from the 75 percentile (BIW≥1.54 kg) and, finally, the MBIW included piglets between 1.20-1.5 kg.

At the average age of 24 days-old, 184 female and 175 male piglets were weaned. After 132 females and 132 males (16 VLBIW, 24 LBIW, 55 MBIW and 37 HBIW pigs for each sex) were randomly selected and housed during the transition phase in groups of 12 piglets/pen distributed by sex and BIW. During the growing-fattening phase (from 72 days-old to the slaughter), groups were randomly equaled to 120 males and 120 females (16 VLBIW, 24 LBIW, 44 MBIW and 36 HBIW pigs for each sex). Finally, 117 males and 115 females were slaughtered.

*Evaluation of litter and piglet data at birth*

Three litter size categories were defined: small litters (3-6 total piglets), medium litters (7-9 total piglets), and large litters (10-13 total piglets). Furthermore, the CV and the SD of BIW was calculated using the BIW of total piglets born alive for each litter.

*Evaluation of growth pattern and fatness*

Samplings were carried out from birth to the slaughter at the following time points: birth, weaning (24 days-old), at six time-periods during postnatal growth (D 71, 110, 150, 180, 215 and 240 of average age) and at slaughter whenever they reached the minimum market-weight (115 kg carcass weight; a time range from 240 to 340 days-old). Body weight was determined individually at all these time points. At 340 days-old, the remaining pigs were sent to market independently of their weight.

Average daily weight gain (**ADWG**) was calculated individually for the suckling phase, the transition phase (25-71 days-old), five periods in the growing-fattening phase (72-110 d, 111-150, 151-180, 181-215 and 216-240 days of age; each period being named by its last day) and the whole productive life. Feed conversion rate (**FCR**) for the five selected periods of the growing-fattening phase was calculated using the formula: daily feed intake block mean/ADWG of the period.

At birth and at weaning, morphological measures were recorded with measuring tape. At weaning and at 110 and 215 days-old, backfat depth was determined at the level of the head of the last rib (P2 point), with a ultrasound machine (SonoSite Inc., USA). At the slaughterhouse, the length of carcasses and the back-fat thickness (at the last rib) were recorded. Carcass yield was calculated individually.

*Tissue sample collection and drip-loss analysis*

Samples of *Longissimus dorsi* (**LD**), of the right lateral lobe of the liver and subcutaneous fat (**SCF**) at the measure level were biobanked at –20 °C until FA analysis. On the same sampling day, a sample of LD muscle was used for the drip-loss analysis carrying out (Calvo *et al.* 2016).

*Evaluation of metabolic status*

Metabolic status was assessed at slaughtering by plasma blood samples obtained by jugular puncture with 5 ml sterile heparin vacuum-tubes (Vacutainer Systems Europe, France). Plasma was separated and stored in vials at -20°C until assayed. Parameters were assessed with a clinical chemistry analyzer (Crony Instruments s.r.l., Italy). Plasma insulin concentrations were also determined, using a Porcine ELISA kit (Mercodia, Sweden), in slaughter samples.

*Evaluation of the fatty acid composition of the diets*

The one-step procedure proposed by Sukhija and Palmquist (1988) was used for the extraction and methylation of the diet FA. Fatty acid methyl esters were identified by a gas chromatograph (Hewlett Packard HP-6890, USA) with a flame ionization detector and a capillary column (HP-Innowax, 30 m × 0.32 mm i.d. and 0.25 µm polyethylene glycol-film thickness; Lopez-Bote *et al.* 1997).

*Evaluation of fat content and fatty acid composition of tissue samples*

The lipids from intramuscular fat at LD muscle (**IMF**) and liver fat were extracted as described by Segura and Lopez-Bote (2014). After, total lipids at IMF and liver fat were fractionated into the main fractions (neutral, **NL**, and polar lipids, **PL**) (Ruiz *et al.* 2004). Subcutaneous fat was individually analyzed in outer and inner layers. Extracts were methylated (Segura *et al.* 2015b) and analyzed using protocols developed at our laboratory (Lopez-Bote *et al.* 1997). From individual **FA** percentages, the saturated, monounsaturated and polyunsaturated FA (**SFA**, **MUFA** and **PUFA**) proportions were calculated. Moreover, the sum of total n-3 FA and n-6 FA and the ratio of both (∑n-6/∑n-3) were calculated. Finally, the activity of stearoyl-CoA desaturase enzyme 1 (**SCD1**) and the unsaturated index (**UI**) were calculated (Hulbert *et al.* 2007). The SCD1 activity was estimated as C18:1/C18:0 and MUFA/SFA ratios, the desaturation indexes (Hulver *et al.* 2005).

*Statistical analysis*

Data were analyzed using the GLM procedure contained in the SAS version 9.4 (Statistical Analysis System Institute Inc., USA) with orthogonal contrasts (5 contrasts). The first contrast was between sexes, the second was between VLBIW pigs and the other BIW groups, the third was between VLBIW and LBIW pigs, the fourth was between LBIW pigs and the sum of MBIW and HBIW pigs and the fifth contrast was between MBIW and HBIW pigs. Birth-weight groups and sex were considered as main effects. Statistically significant interactions were indicated in each table of results. Sow was used as block in birth and weaning analysis to account for the common maternal environment. Litter size was categorized into the three groups previously described and used as a random effect for birth-data. For performance parameters, the respective age was used as a covariate. Moreover, Duncan's test was used to identify differences between groups. Chi-square was used to assess the mortality data and the percentage of VLBIW piglets, as described above. Pearson's correlations were analyzed using the PROC CORR and regressions using the PROC REG procedures of SAS. The pig was the experimental unit for all the variables studied except for the CV and SD of BIW and the LS where sow was the unit and for the FCR data with the pen. All the results were expressed as mean ± RMSE in tables, but mean ± SD was used for figures and text. Statistical significance was accepted from $P<0.05$.

## Results

*Litter size and morphometric data of newborns*

The mean LS was 8.9±2.6 total piglets born per sow and distributed in 8.6±2.6 piglets born alive. The incidence of VLBIW and LBIW piglets were 15.8% and 14% of the total piglets born alive, respectively. There were no significant differences between male and female sexes in mean BIW (1.31±0.34 vs. 1.32±0.29 kg) or incidence of smaller piglets (16.3 vs. 13.3%, respectively, for VLBIW and 15.3 vs. 12.8%, respectively, for LBIW). The mean BIW was significantly related to all morphological measures ($P$<0.0001; T1) and a longer thoracic perimeter in female than in male piglets (23.9±2.1 cm in females vs. 23.4±2.5 cm in males, $P$<0.005) was the only sex-related effect.

The 48.9% of the litters were classified as high prolific, while 32% and 19.1% were medium and low prolific, respectively. Moreover, the higher LS, the more LBIW piglets were found for both sexes ($P$<0.001).

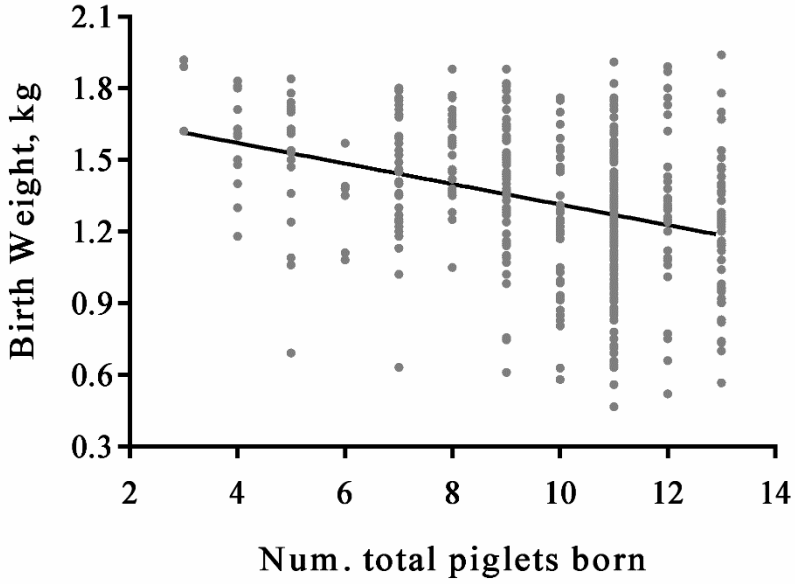


**Fig. 1.** Regression of number total piglets born per litter and birth-weight. Equation: BIW = (1.74±0.06) – (0.043±0.006)PB. $R^2$ = 0.11, rSD = 0.29, P<0.0001. PB= Piglets born per litter, BIW= Birth-weight.

Therefore, the mean value of BIW was lowered with higher prolificacy (1.51±0.26 kg in low LS vs. 1.41±0.28 kg in medium LS vs. 1.23±0.31 kg in high LS, $P$<0.0001). Consequently, mean BIW decreased by approximately 43 g per pig with each unit increases in the number of total piglets born ($P$<0.001; Fig. 1). The within-litter BIWV, measured on the basis of CV, was higher with higher LS (r=0.45, $P$<0.005; T1) and there were found significant

differences between the three categories (12.1±8.4% in low LS vs. 18.4±6.4% in medium LS vs. 22.0±4.7% in high LS, $P<0.001$). These data were reinforced when analyzing BIWV by SD (0.18±0.10 in low LS vs. 0.25±0.08 in medium LS vs. 0.27±0.06 in high LS, $P<0.01$).

*Effects of birth-weight and sex on weaning traits*

A mean of 7.7±0.7 piglets was weaned per sow, with a mean total litter weight of 42.1±7.6 kg. Overall, without significant differences between sexes and there was 11.5% of mortality during the suckling phase. The BIW showed a dramatic influence on mortality, being higher in the VLBIW group (36% vs. 7% in the other groups; $P<0.0001$).

The mean body weight at weaning was significantly correlated with BIW (r=0.50, $P<0.0001$; T2) and all morphological measures were significantly different between BIW categories ($P<0.05$, for all; Table 1). The lowest measures and weights were found in the VLBIW group, with significant differences comparing to the other groups (LBIW, MBIW and HBIW; $P<0.001$, for all). Moreover, males showed thicker backfat depth than females (0.48±0.11 cm vs. 0.44±0.12 cm, $P<0.01$).

The VLBIW piglets also showed a lower ADWG during the suckling phase (0.14±0.04 kg/d) than the other groups (mean value of 0.19±0.05 kg/d; $P<0.0001$), and the LBIW group showed lower ADWG than piglets in groups MBIW and HBIW ($P<0.05$).

**Table 1.** Phenotypic parameters at birth and weaning.

| | | Groups | | | | | | | | | | | | | | *P*-values Contrast | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | VLBIW | | | | LBIW | | | | MBIW | | | | HBIW | | | | | | | |
| Item | *n* | Females | | Males | | Females | | Males | | Females | | Males | | Females | | Males | | RMSE | 2 | 3 | 4 | 5 | Int |
| Birth | | | | | | | | | | | | | | | | | | | | | | | |
| Body Wt, kg | 406 | 0.8 | D | 0.8 | D | 1.1 | C | 1.1 | C | 1.4 | B | 1.4 | B | 1.7 | A | 1.7 | A | 0.1 | <.0001 | <.0001 | <.0001 | <.0001 | <.0001 |
| Occipito-nasal L. | 406 | 11.9 | D | 11.6 | D | 12.3 | C | 12.4 | BC | 12.7 | B | 12.6 | B | 13.0 | A | 13.1 | A | 0.6 | <.0001 | <.0001 | <.0001 | <.0001 | <.0001 |
| Trunk L. | 406 | 19.6 | E | 19.3 | D | 21.5 | C | 21.3 | C | 22.8 | B | 22.8 | B | 24.5 | A | 24.4 | A | 1.4 | <.0001 | <.0001 | <.0001 | <.0001 | <.0001 |
| Abdominal Perim. | 406 | 15.7 | D | 15.1 | D | 17.1 | C | 17.3 | C | 18.5 | B | 18.5 | B | 20.3 | A | 20.0 | A | 1.3 | <.0001 | <.0001 | <.0001 | <.0001 | <.0001 |
| Thoracic Perim. | 406 | 20.2 | D | 19.5 | E | 22.5 | C | 22.2 | C | 24.4 | B | 24.1 | B | 26.1 | A | 25.9 | A | 1.2 | <.0001 | <.0001 | <.0001 | <.0001 | <.0001 |
| Biparietal Diam. | 406 | 4.7 | D | 4.6 | D | 5.1 | C | 5.1 | C | 5.3 | B | 5.3 | B | 5.6 | A | 5.6 | A | 0.2 | <.0001 | <.0001 | <.0001 | <.0001 | <.0001 |
| Maximum Thoracic Diam. | 406 | 5.6 | D | 5.5 | D | 6.4 | C | 6.4 | C | 6.9 | B | 6.8 | B | 7.4 | A | 7.4 | A | 0.5 | <.0001 | <.0001 | <.0001 | <.0001 | <.0001 |
| Weaning | | | | | | | | | | | | | | | | | | | | | | | |
| Body Wt, kg | 359 | 4.2 | C | 4.2 | C | 5.2 | B | 5.3 | B | 5.7 | AB | 5.5 | B | 6.3 | A | 6.3 | A | 1.2 | <.0001 | <.0001 | 0.0003 | <.0001 | <.0001 |
| Occipito-nasal L. | 359 | 15.2 | C | 15.1 | C | 16.0 | B | 15.8 | B | 16.3 | AB | 16.3 | A B | 16.5 | A | 16.6 | A | 1.0 | <.0001 | 0.0004 | 0.0007 | 0.03 | <.0001 |
| Trunk L. | 359 | 35.1 | D | 34.8 | D | 38.8 | C | 38.5 | C | 40.2 | BC | 39.5 | BC | 42.3 | A | 41.3 | A B | 4.0 | <.0001 | <.0001 | 0.0004 | 0.0002 | <.0001 |
| Abdominal Perim. | 359 | 30.5 | C | 30.4 | C | 33.2 | B | 33.1 | B | 34.3 | AB | 33.9 | B | 36.2 | A | 35.1 | A B | 3.8 | <.0001 | 0.0007 | 0.003 | 0.001 | <.0001 |
| Thoracic Perim. | 359 | 35.2 | C | 34.2 | C | 37.2 | B | 37.3 | B | 38.9 | AB | 38.2 | B | 40.6 | A | 40.0 | A | 3.4 | <.0001 | 0.0004 | <.0001 | 0.0001 | <.0001 |
| ADWG, kg/d | 359 | 0.1 | C | 0.1 | C | 0.2 | B | 0.2 | A B | 0.2 | AB | 0.2 | A B | 0.2 | A | 0.2 | A | 0.0 | <.0001 | <.0001 | 0.01 | 0.002 | <.0001 |

VLBIW= Very low birth-Wt, LBIW= Low birth-Wt, MBIW= Medium birth-Wt, HBIW= High birth-Wt. L= Length, Perim.= Perimeter, ADWG= Average daily weight gain. Wt=Weight. RMSE = root-mean-square error. Ns= not significant, t= 0.1>*P*>0.05. L., Perim. and Diam. in cm.
Different letters in a line indicate significant differences (P<0.05). C2: VLBIW-(LBIW+MBIW+HBIW); C3: VLBIW-LBIW; C4: LBIW-(MBIW+HBIW); C5: MBIW-HBIW; Int: Interaction birth-Wt and sex.

**Table 2.** Correlation coefficients comparing litter size, within-litter variation, weights and carcass traits.

| | | WT, kg | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Item | LS | Birth | Weaning | 71 d | 110 d | 150 d | 180 d | 215 d | 240 d | DaM, d | Carcass yield, % | Backfat depth, cm | IMF, % |
| CV of Birth WT | 0.4 ** | -0.3 ** | 0.0 | 0.0 | 0.1 | 0.0 | -0.1 | -0.1 | -0.1 | 0.1 t | -0.1 | -0.1 | -0.1 t |
| LS | | -0.3 ** | -0.1 * | 0.0 | 0.1 | 0.0 | 0.0 | -0.1 | -0.1 | 0.2 * | 0.1 | 0.1 | 0.0 |
| WT, birth | | | 0.5 *** | 0.2 ** | 0.1 | 0.1 | 0.1 | 0.1 | 0.2 * | -0.3 *** | -0.2 * | -0.1 | -0.2 ** |
| WT, Weaning | | | | 0.6 *** | 0.5 *** | 0.5 *** | 0.4 *** | 0.3 *** | 0.3 *** | -0.3 *** | -0.2 * | -0.2 * | -0.2 * |
| WT, 71 d | | | | | 0.8 *** | 0.8 *** | 0.7 *** | 0.6 *** | 0.6 *** | -0.4 *** | -0.2 ** | -0.1 | -0.1 |
| WT, 110 d | | | | | | 0.8 *** | 0.7 *** | 0.6 *** | 0.6 *** | -0.4 *** | -0.3 ** | -0.1 | -0.1 |
| WT, 150 d | | | | | | | 0.9 *** | 0.9 *** | 0.8 *** | -0.6 *** | -0.3 ** | -0.1 | 0.0 |
| WT, 180 d | | | | | | | | 0.9 *** | 0.8 *** | -0.6 *** | -0.3 ** | 0.0 | 0.0 |
| WT, 215 d | | | | | | | | | 0.9 *** | -0.7 *** | -0.3 ** | 0.0 | 0.0 |
| WT, 240 d | | | | | | | | | | -0.8 *** | -0.3 ** | 0.0 | 0.0 |
| DaM, d | | | | | | | | | | | 0.4 ** | 0.0 | 0.0 |
| Carcass yield, % | | | | | | | | | | | | 0.2 ** | 0.1 * |
| Backfat depth, cm | | | | | | | | | | | | | 0.2 * |

LS= litter size, DaM= days to market, IMF= Intramuscular muscular fat. WT=Weight. Asterisks indicate significant differences between groups (t= 0.1>*P*>0.05, *=*P*<0.05, **=*P*<0.005, ***=*P*<0.0001).

*Effects of birth weight and sex on growing and fattening phases*

At the end of the growth-transition phase (71 days-old), VLBIW pigs continued being lighter than the pigs in the other categories (LBIW, MBIW and HBIW; $P<0.001$; Fig. 2A-B) and showing lower values of ADWG (0.34±0.08 kg/d vs. 0.38±0.08 kg/d, respectively; $P<0.01$). There were also similar effects in LBIW pigs with lower weight and ADWG than pigs of the heaviest BIW groups (MBIW and HBIW; $P<0.001$ and $P<0.05$, respectively; T3).

In spite of these effects, the mean weight at the end of the transition phase was weakly related to BIW (T2) although strongly correlated with the weaning weight (r=0.61; P<0.0001). In the same way, BIW showed no correlation with body weights at the different periods assessed during the growing-fattening phases (110, 150, 180, 215 and 240 days of average age). However, it showed a positive correlation with age at market (r=0.32, $P<0.0001$).

At the beginning of the growing phase, at 110 days-old, HBIW category showed a higher mean body weight than the MBIW category ($P<0.0001$; Fig. 2A-B, T3) and LBIW pigs were heavier than VLBIW pigs ($P<0.01$). Furthermore, MBIW pigs showed lower ADWG and higher FCR than HBIW pigs ($P<0.01$, for both), while both HBIW and MBIW also showed lower ADWG and higher FCR than LBIW pigs ($P<0.005$, for both, T3). There was a significant sex-related effect in all the groups since females showed greater weight and ADWG and lower FCR than males ($P<0.01$, for all groups).

At the following assessment, 150 days-old, HBIW pigs still maintained a higher weight than MBIW pigs ($P<0.05$, T3). Pigs categorized as VLWB pigs remained lighter than the pigs in the other groups (54.0±11.4 kg in the VLBIW group vs. 58.7±9.1 kg in the other groups, $P<0.001$), mainly due to the lowest weight of VLBIW females at 150 days-old (P<0.0001; Fig. 2A-B). At this age, the VLBIW category also showed lower ADWG and higher FCR than the other categories ($P<0.0005$, for both; T3). At 180 days-old, VLBIW pigs continued having less weight and ADWG than the pigs of the other groups at the next period ($P<0.05$) and FCR was lower with heavier BIW ($P<0.001$). Regarding the sex-effect, at 150 days-old, females had less ADWG and more FCR than males ($P<0. 0005$, for both). However, at 180 days-old females showed less weight and ADWG than males ($P<0.001$, for both).

With regards to the fattening phase, the VLBIW group remained with lower weight and ADWG, at both 215 and 240 days-old, and greater FCR, at 215 days-old, than the other groups ($P<0.05$, for all; T3 and Fig. 2A-B). At 240 days-old, the LBIW group also showed

lower ADWG and higher FCR than HBIW and MBIW groups (*P*<0.005, for both; T3), mainly because LBIW males showed higher FCR. Moreover, all males showed greater weight and ADWG than females at 240 days-old.

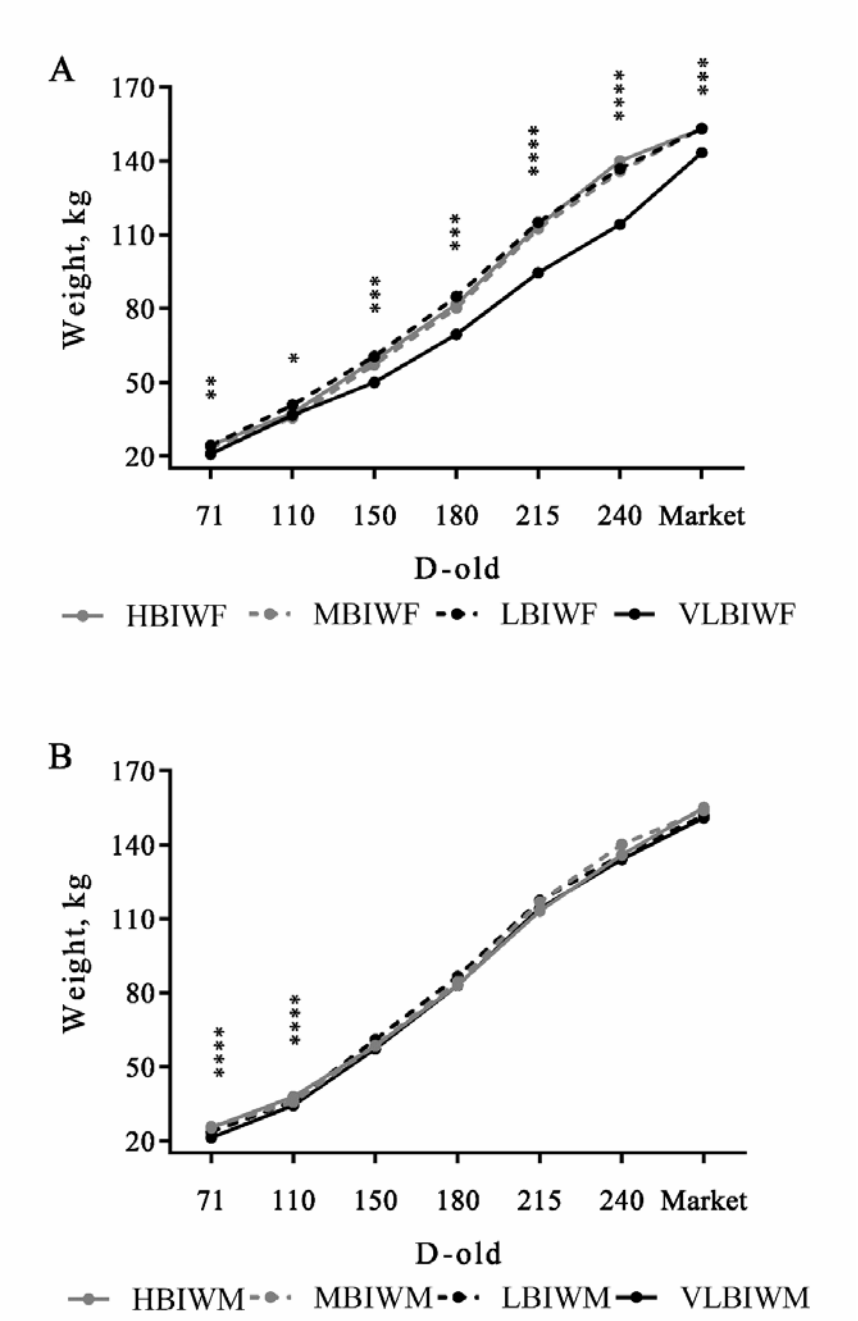


**Fig. 2.** Weight evolution during growing and fattening phases until at market. In females (Pannel A) and males (B) distributed by birth-weight (BIW) categories. Asterisks indicate significant differences between BIW groups separated by sex (*=P<0.05, **=P<0.005, ***=P<0.001, ****=P<0.0001).

**Table 3.** Growth during growing and fattening phases and at market

| | | Groups | | | | | | | | | | | | | *P*-value Contrasts | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | VLBIW | | LBIW | | MBIW | | HBIW | | | | | | | | | | | | |
| Item | *n* | Females | Males | Females | Males | Females | Males | Females | Males | RMSE | 1 | 2 | 3 | 4 | 5 | Int |
| 71 d-old | | | | | | | | | | | | | | | | |
| Body Wt, kg | 258 | 20.9 $^{B}$ | 21.3 $^{B}$ | 24.2 $^{A}$ | 23.6 $^{A}$ | 23.6 $^{A}$ | 25.7 $^{A}$ | 24.4 $^{A}$ | 25.2 $^{A}$ | 3.75 | ns | <.0001 | 0.0007 | 0.001 | 0.02 | <.0001 |
| ADWG, kg/d | 258 | 0.3 $^{B}$ | 0.3 $^{B}$ | 0.4 $^{AB}$ | 0.4 $^{AB}$ | 0.4 $^{AB}$ | 0.4 $^{A}$ | 0.4 $^{A}$ | 0.4 $^{A}$ | 0.07 | ns | <.0001 | 0.007 | 0.04 | ns | <.0001 |
| 110 d-old | | | | | | | | | | | | | | | | |
| Body Wt, kg | 240 | 36.7 $^{BC}$ | 34.3 $^{C}$ | 40.7 $^{A}$ | 36.1 $^{BC}$ | 35.6 $^{BC}$ | 35.8 $^{BC}$ | 37.5 $^{ABC}$ | 37.8 $^{AB}$ | 5.25 | 0.008 | 0.002 | 0.008 | ns | <.0001 | <.0001 |
| ADWG, kg/d | 240 | 0.4 $^{A}$ | 0.4 $^{B}$ | 0.5 $^{A}$ | 0.3 $^{B}$ | 0.3 $^{BC}$ | 0.3 $^{C}$ | 0.4 $^{B}$ | 0.4 $^{B}$ | 0.09 | <.0001 | t | ns | 0.0002 | 0.0001 | <.0001 |
| FCR, kg/kg | 240 | 1.0 $^{C}$ | 1.3 $^{B}$ | 0.9 $^{C}$ | 1.4 $^{AB}$ | 1.5 $^{AB}$ | 1.7 $^{A}$ | 1.4 $^{AB}$ | 1.4 $^{AB}$ | 0.52 | 0.0007 | t | ns | 0.002 | 0.009 | <.0001 |
| 150 d-old | | | | | | | | | | | | | | | | |
| Body Wt, kg | 238 | 49.9 $^{B}$ | 57.3 $^{A}$ | 60.6 $^{A}$ | 61.0 $^{A}$ | 57.1 $^{A}$ | 58.6 $^{A}$ | 58.8 $^{A}$ | 58.4 $^{A}$ | 7.46 | ns | <.0001 | <.0001 | ns | 0.01 | 0.0003 |
| ADWG, kg/d | 238 | 0.4 $^{D}$ | 0.7 $^{AB}$ | 0.6 $^{C}$ | 0.7 $^{A}$ | 0.6 $^{BC}$ | 0.7 $^{AB}$ | 0.6 $^{BC}$ | 0.6 $^{BC}$ | 0.13 | <.0001 | <.0001 | 0.0004 | ns | ns | <.0001 |
| FCR, kg/kg | 238 | 3.6 $^{A}$ | 2.0 $^{B}$ | 2.1 $^{B}$ | 1.8 $^{B}$ | 1.9 $^{B}$ | 1.9 $^{B}$ | 2.2 $^{B}$ | 2.1 $^{B}$ | 0.83 | 0.0002 | <.0001 | 0.0001 | ns | ns | <.0001 |
| 180 d-old | | | | | | | | | | | | | | | | |
| Body Wt, kg | 237 | 69.4 $^{C}$ | 83.0 $^{AB}$ | 84.8 $^{AB}$ | 86.6 $^{A}$ | 80.2 $^{B}$ | 84.4 $^{AB}$ | 81.7 $^{AB}$ | 83.1 $^{AB}$ | 9.58 | 0.0006 | <.0001 | <.0001 | ns | ns | <.0001 |
| ADWG, kg/d | 237 | 0.6 $^{C}$ | 0.7 $^{A}$ | 0.7 $^{AB}$ | 0.7 $^{A}$ | 0.7 $^{B}$ | 0.7 $^{A}$ | 0.7 $^{B}$ | 0.7 $^{AB}$ | 0.11 | <.0001 | 0.02 | 0.01 | ns | ns | <.0001 |
| FCR, kg/kg | 237 | 5.0 $^{A}$ | 4.1 $^{B}$ | 3.8 $^{B}$ | 4.1 $^{B}$ | 4.0 $^{B}$ | 3.9 $^{B}$ | 3.1 $^{C}$ | 3.2 $^{C}$ | 0.72 | ns | <.0001 | 0.001 | 0.0004 | <.0001 | <.0001 |
| 215 d-old | | | | | | | | | | | | | | | | |
| Body Wt, kg | 234 | 94.5 $^{B}$ | 114.2 $^{A}$ | 115.0 $^{A}$ | 117.4 $^{A}$ | 112.4 $^{A}$ | 116.7 $^{A}$ | 113.5 $^{A}$ | 113.1 $^{A}$ | 11.88 | 0.0006 | <.0001 | <.0001 | ns | ns | <.0001 |
| ADWG, kg/d | 234 | 0.7 $^{B}$ | 0.9 $^{A}$ | 0.9 $^{A}$ | 0.9 $^{A}$ | 0.9 $^{A}$ | 0.9 $^{A}$ | 0.9 $^{A}$ | 0.9 $^{A}$ | 0.14 | ns | 0.02 | ns | ns | t | 0.01 |
| FCR, kg/kg | 234 | 4.4 $^{A}$ | 4.2 $^{AB}$ | 3.8 $^{B}$ | 4.2 $^{AB}$ | 3.7 $^{B}$ | 3.9 $^{AB}$ | 3.8 $^{B}$ | 4.2 $^{AB}$ | 0.83 | ns | 0.03 | ns | ns | ns | 0.04 |
| 240 d-old | | | | | | | | | | | | | | | | |
| Body Wt, kg | 232 | 114.2 $^{B}$ | 134.1 $^{A}$ | 136.8 $^{A}$ | 135.8 $^{A}$ | 135.7 $^{A}$ | 140.1 $^{A}$ | 140.0 $^{A}$ | 135.9 $^{A}$ | 13.63 | 0.03 | <.0001 | 0.0004 | ns | ns | <.0001 |

| | | | | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| ADWG, kg/d | 232 | 0.8 | CD | 0.8 | CD | 0.9 | BC | 0.7 | D | 0.9 | B | 0.9 | BC | 1.1 | A | 0.9 | BC | 0.20 | 0.02 | 0.02 | ns | 0.0001 | ns | <.0001 |
| FCR, kg/kg | 232 | 5.9 | AB | 5.5 | ABC | 5.2 | ABCD | 6.5 | A | 4.2 | DC | 4.2 | DC | 4.0 | D | 4.7 | BCD | 1.67 | ns | t | ns | 0.002 | ns | <.0001 |
| Slaughter | | | | | | | | | | | | | | | | H | | | | | | | | |
| Body Wt, kg | 232 | 143.3 | B | 151.0 | A | 153.1 | A | 151.9 | A | 153.4 | A | 153.9 | A | 153.0 | A | 155.1 | A | 7.53 | 0.02 | <.0001 | 0.003 | ns | ns | ns |
| DaM, d | 232 | 306.3 | A | 284.7 | B | 274.5 | BC | 283.8 | B | 271.1 | BC | 273.2 | BC | 262.7 | C | 268.7 | C | 22.12 | ns | <.0001 | 0.004 | 0.009 | t | ns |
| ADWG, kg/d | 232 | 0.5 | D | 0.5 | BC | 0.6 | ABC | 0.5 | BC | 0.6 | ABC | 0.6 | ABC | 0.6 | A | 0.6 | AB | 0.06 | ns | <.0001 | 0.0009 | 0.04 | ns | ns |

VLBIW= Very low birth-Wt, LBIW= Low birth-Wt, MBIW= Medium birth-Wt, HBIW= High birth-Wt. ADWG= Average daily weight gain, DaM= days to market. Wt=Weight. RMSE = root-mean-square error. Ns= not significant, t= 0.1>P>0.05.

Different letters in a line indicate significant differences (P<0.05). Contrast 1: Females-Males; C2: VLBIW-(LBIW+MBIW+HBIW); C3: VLBIW-LBIW; C4: LBIW-(MBIW+HBIW); C5: MBIW-HBIW; Int: Interaction birth-Wt and sex.

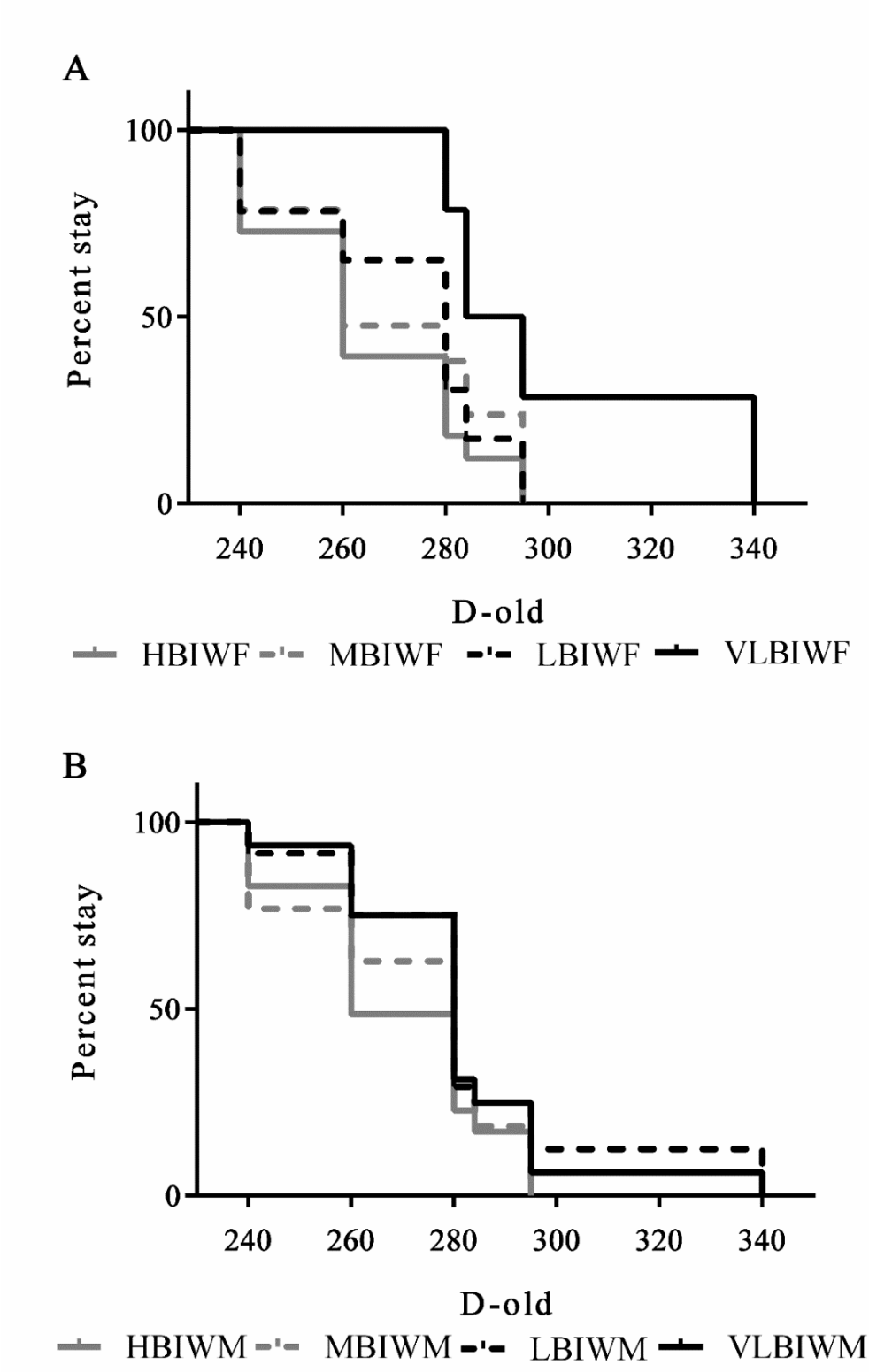


**Fig. 3.** Percentage of pig stay at farm since the first pigs go at market. In females (Pannel A) and males (B) distributed by birth-weight (BIW) categories.

At slaughter, the assessment of the ADWG for the overall period revealed that the VLBIW group had lower values than the other groups (0.51±0.07 kg/d in the VLBIW group vs. 0.57±0.06 kg/d in the other groups, $P<0.001$). Moreover, LBIW pigs also showed less total ADWG than the heaviest BIW pigs ($P<0.05$; T3). The average of days to market (**DaM**) linearly decreased with BIW increase ($P<0.05$; T4 and Fig. 3A-B), so DaM was negatively correlated with birth and postnatal weights (T2) and decreased by approximately 28 days per kg of BIW increased ($P<0.0001$; T4). Hence, VLBIW pigs were the oldest group to be slaughtered but, moreover, the VLBIW group was the lightest one at slaughter. Furthermore, VLBIW females were the lightest individuals at slaughter ($P<0.0001$; T3).

**Table 4.** Regression equation between days to market (d), birth-weight (kg), ADWG (kg/d) and weight at slaughter (kg).

| | | | | *P*-value | | |
|---|---|---|---|---|---|---|
| Item | n | $R^2$ | rSD | Linear | Quadratic | Regression |
| DaM = (311.7±7.4) – (27.6±5.3) BIW | 232 | 0.11 | 22.4 | - | - | 0.0001 |
| ADWG = (1.25±0.02) – (0.003±0.00007) DaM | 232 | 0.86 | 0.024 | - | - | 0.0001 |
| WM = (55.2±36.6) + (0.85±0.002) DaM – (0.002±0.0005) $DaM^2$ | 232 | 0.25 | 6.15 | 0.002 | 0.0003 | 0.0001 |
| DaM (males) = ($305.2^b$±9.6) – ($22.2^b$±6.9) BIW | 117 | 0.08 | 21.97 | - | - | 0.0001 |
| DaM (females) =($320.5^a$±11.6) – ($34.9^a$ ±8.3) BIW | 115 | 0.14 | 23.02 | - | - | 0.0001 |

DaM= Days to market, BIW= Birth-weight, WM= weight at market, ADWG= average daily weight gain. According to sex, using Student test, intercepts and slopes with different superscripts are different ($P<0.05$).

The simple linear regression relationships between DaM and BIW (T4), compared by means according to BIW categories and its interaction with sex, were not significant. Regarding sex, intercepts and slopes of the regression equations of the relationship between BIW and DaM were different ($P<0.05$). Moreover, ADWG linearly decreased with increasing DaM. Days to market also accounted for 25% of the weight at market variation and the maximum point calculated was 238 d, which means that above this value weight at market decreased as increasing DaM.

*Effect of birth weight and sex on metabolic status*

At slaughter, higher glucose concentrations were found in VLBIW pigs (127.3±23.4 mg/dL) than in the other groups (mean value of 107.6±28.8 mg/dL; $P<0.05$), with a significant sex-related effect since VLBIW females showed the highest values (153.0±14.1 mg/dL). On the other hand, plasma concentrations of insulin were the highest in VLBIW males and the lowest in VLBIW females (0.03±0.00 µg/L for females vs. 0.09±0.05 for males; $P<0.05$).

*Effect of birth weight and sex on carcass traits and fatness*

There were significant differences in fat accumulation between groups during postnatal development. The VLBIW group showed smaller backfat depth than the other groups at weaning and at 110 and 215 days-old. (Fig. 4; $P<0.05$). Differentiation of inner and outer layers at the last two stages indicated that both were thinner in the VLBIW group than the in other groups at 110 days-old ($P<0.005$ and $P<0.05$ for inner and outer layers, respectively) while only the outer layer was found to be significantly lower at 215 days-old ($P<0.05$). At 110 days-old, the outer layer was thinner in MBIW pigs than in HBIW pigs ($P<0.05$). Moreover, the LBIW group also showed thicker total backfat and inner layer than the heaviest BIW groups at 215 days-old ($P<0.05$, for both).

Total backfat depth was thicker in males than in females at weaning ($P<0.01$) while, at 110 days-old, male pigs had thicker inner layer, but thinner outer layer than female pigs ($P<0.05$, for both). At 215 days-old, males continued having thicker total back-fat and inner layer than females ($P<0.0005$, for both). Conversely, at slaughter, there were not significant differences in back-fat depth of carcasses between groups or between sexes (T5).

Regarding carcass traits at market, carcass length was shorter in the VLBIW category than in the other categories ($P<0.05$; T5) with VLBIW females having the lowest carcass length and weight. The carcass yield was positively correlated with the age at market (T2) and the LBIW group showed greater IMF than the heaviest BIW groups ($P<0.05$). Sex effects were found for IMF and liver fat, which were higher in males than in females ($P<0.05$, for both; T5).

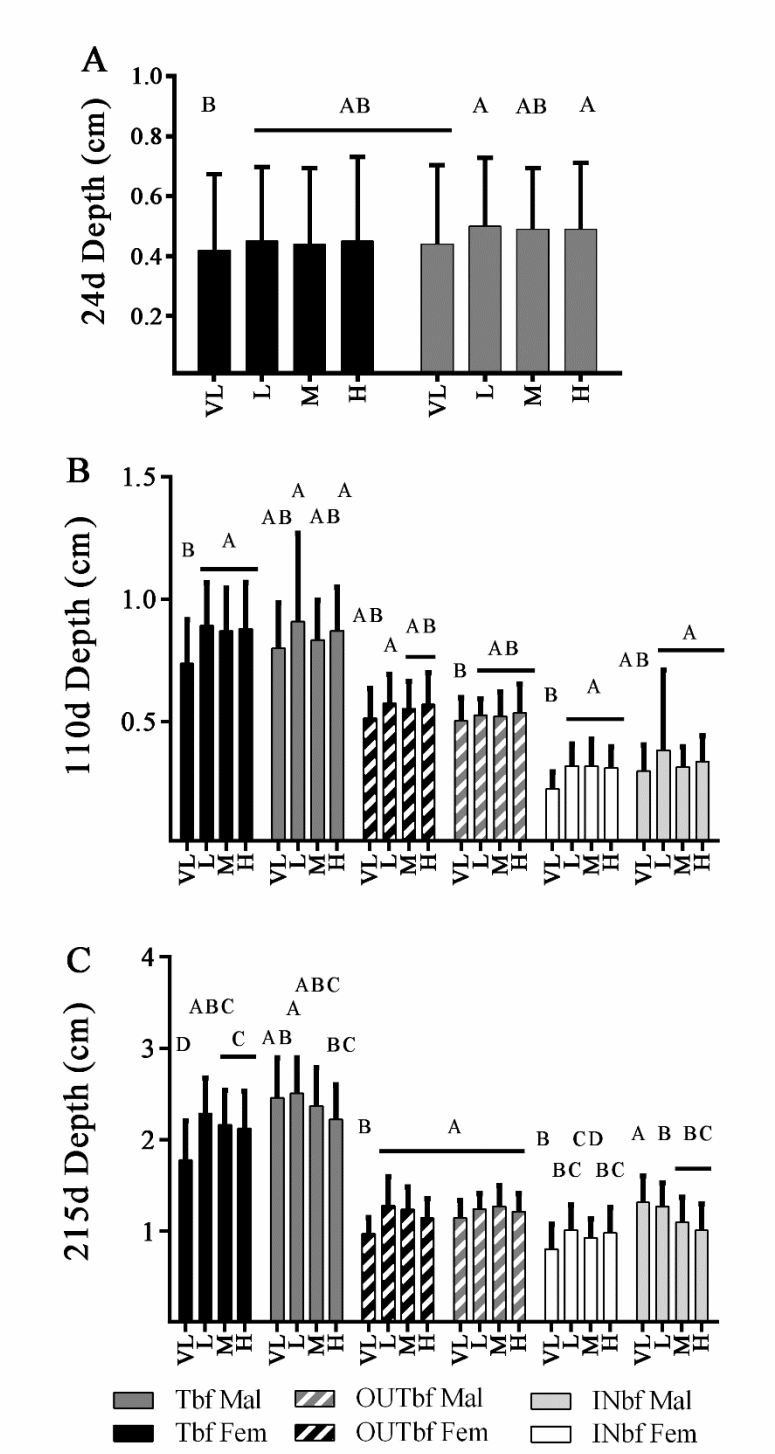


**Fig. 4.** Fatness during postnatal development. Mean values (±SD) of total backfat depth (Tbf, cm) and its outer and inner layers (OUTbf and INbf) in females (Fem) and males (Mal) distributed by birth-weight categories at weaning (Pannel A ), at 110 days-old (Pannel B) and at 215 days-old (Pannel C). Different letters indicate significant differences by Duncan's test between all groups.The BIW categories are very low BIW (VL), low BIW (L), medium BIW (M) and high BIW (H).

**Table 5.** Carcass and meat quality traits at market

| | | Groups | | | | | | | | | | | | *P*-value Contrasts | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | VLBIW | | LBIW | | MBIW | | HBIW | | | | | | | | | | |
| Item | *n* | Females | Males | Females | Males | Females | Males | Females | Males | RMSE | 1 | 2 | 3 | 4 | Int |
| Carcass Wt, kg | 232 | 118.0 $^{B}$ | 121.6 $^{AB}$ | 122.3 $^{AB}$ | 123.2 $^{A}$ | 122.1 $^{AB}$ | 121.9 $^{AB}$ | 121.6 $^{AB}$ | 122.0 $^{AB}$ | 6.2 | ns | ns | ns | ns | ns |
| Carcass yield, % | 232 | 79.0 | 79.6 | 79.9 | 79.6 | 79.3 | 79.3 | 78.6 | 78.8 | 2.2 | ns | ns | ns | ns | ns |
| Carcass length, cm | 232 | 88.1 $^{B}$ | 88.2 $^{AB}$ | 90.4 $^{AB}$ | 89.1 $^{AB}$ | 90.7 $^{AB}$ | 90.0 $^{AB}$ | 90.4 $^{AB}$ | 90.8 $^{A}$ | 2.7 | ns | 0.002 | 0.03 | ns | 0.01 |
| Backfat depth, cm | 232 | 5.3 | 5.3 | 5.2 | 5.2 | 5.2 | 5.2 | 5.0 | 5.1 | 0.7 | ns | ns | ns | ns | ns |
| Muscular drip loss, % | 232 | 6.9 $^{A}$ | 3.9 $^{B}$ | 6.0 $^{AB}$ | 5.6 $^{AB}$ | 5.2 $^{AB}$ | 5.8 $^{AB}$ | 5.5 $^{AB}$ | 5.4 $^{AB}$ | 2.9 | ns | ns | ns | ns | ns |
| Intramuscular fat, % | 232 | 27.7 $^{AB}$ | 28.0 $^{AB}$ | 25.8 $^{B}$ | 30.1 $^{A}$ | 24.0 $^{B}$ | 26.9 $^{AB}$ | 23.9 $^{B}$ | 25.4 $^{B}$ | 6.1 | 0.04 | ns | ns | 0.02 | 0.03 |
| Liver fat, % | 232 | 18.0 $^{C}$ | 24.5 $^{A}$ | 21.8 $^{AB}$ | 21.9 $^{AB}$ | 20.6 $^{BC}$ | 20.7 $^{BC}$ | 19.4 $^{BC}$ | 20.5 $^{BC}$ | 3.1 | 0.02 | ns | t | ns | ns |

VLBIW= Very low birth-Wt, LBIW= Low birth-Wt, MBIW= Medium birth-Wt, HBIW= High birth-Wt. Wt=Weight. RMSE = root-mean-square error. Ns= not significant, t= 0.1> *P*>0.05.

Different letters in a line indicate significant differences (P<0.05). Contrast 1: Females-Males; C2: VLBIW-(LBIW+MBIW+HBIW); C3: VLBIW-LBIW; C4: LBIW-(MBIW+HBIW); C5: MBIW-HBIW; Int: Interaction birth-Wt and sex.

*Effects of birth weight and sex on tissue fatty acid composition*

There were significant effects of both BIW categories and sex on FA profile of the lipids from IMF, SCF and liver fat (Supplementary Tables 2, 3 and 4, respectively).

The composition of lipids at IMF was driven by both BIW and sex. Especifically in PL fraction, the VLBIW category showed greater C16:0, C18:0, C18:1n-9, SFA and MUFA concentrations than the other categories ($P$<0.05, for all). Very low BIW pigs showed greater content of C16:0, C18:0, C18:1n-9, SFA and MUFA than the other categories ($P$<0.05, for all). Conversely, VLBIW pigs had lower C18:2n-6, C22:4n-6, C22:5n-3, PUFA, n-6 FA and n-3 FA values and UI than the other groups ($P$<0.05, for all). Main differences in the NL fraction were found at heavier categories. In this way, LBIW pigs had higher content C18:3n-3 than heavier pigs ($P$<0.05). The MBIW category showed lower concentrations of C18:1n-9, C18:1n-7 and MUFA ($P$<0.01, for all) and UI and desaturation indexes (C18:1/C18:0 and MUFA/SFA; $P$<0.05, for all) than the HBIW category, but higher values of C14:0, C16:0 and SFA ($P$<0.05, for all). There were found significant sex-related effects at both NL and PL fractions. Males showed, at both NL and PL fractions, higher C17:0 concentrations and desaturation indexes but lower C16:0 values than females ($P$<0.05, for all). Considering only the NL fraction, males also had higher concentrations of C16:1n-7, C17:1, C18:1n-7, C18:3n-3 and MUFA and higher UI ($P$<0.05, for all), but lower C18:0 and SFA values ($P$<0.05, for both) than females. At the PL fraction, males showed higher C18:1n-9 content ($P$<0.05).

The assessment of the FA profile at SCF addressed mostly sex-related differences, excepting that, independently from sex, the HBIW group showed higher C18:3n-3 and n-3 FA values than the MBIW group in the outer layer of SCF ($P$<0.05, for both). Females had, in both the outer and inner layers, higher C16:0, C18:0 and SFA concentrations ($P$<0.05, for all), but lower C17:0, C18:1n-9 and MUFA values and desaturation indexes ($P$<0.005 for all) and lower UI ($P$<0.05), than males. Finally, females also showed a higher ∑n-6/∑n-3 ratio in the outer layer ($P$<0.01), with the same amount of n-3 FA, and lower C18:3n-3 and n-3 FA values at the inner layer ($P$<0.005, for both).

The effects of BIW and sex were differently driven at the liver, evidencing more significant differences between BIW groups at NL fraction and more sex-related effects at PL fraction. The assessment of the NL fraction evidenced that the VLBIW group had higher C18:0 and SFA concentrations and lower C18:3n-3 values and desaturation index than the LBIW group ($P$<0.05 for all) with the greatest means of these variables for VLBIW

females. On the other hand, the LBIW group, when compared to MBIW and HBIW groups, had higher C18:1n-9 and MUFA concentrations ($P$<0.05) and desaturation indexes (C18:1/C18:0 and MUFA/SFA; $P$<0.0001), and lower C18:0, C20:5 n-3, C22:6 n-3 and saturated FA (SFA) values than the heaviest BIW categories ($P$<0.05, for all). Finally, the C20:3 n-6 concentration was higher in the HBIW group ($P$<0.05) than in the MBIW group. Effects of sex on FA composition at NL were limited to a higher C18:0 and SFA concentrations in VLBIW females than in all the other groups ($P$<0.05, for both). However, the sex-related effects were critical at the PL fraction, where BIW induced higher values in C20:3 n-6 concentration for HBIW pigs than for MBIW pigs ($P$<0.005) and higher C18:0 levels for the VLBIW group ($P$<0.05) than for the other groups. In fact, female and male VLBIW pigs were the most divergent in FA composition of PL fraction, resulting in many significant global sex effects. Males showed greater C16:0, C17:1, C18:1n-9, C18:1n-7, C18:3n-3, C20:1 n-9 and MUFA concentrations and desaturation indexes ($P$<0.05, for all) than females, but lower C18:0 and C18:2n-6 values ($P$<0.005, for both).

## Discussion

The present study gives comprehensive evidence for first time in a fatty breed that increases in LS like previously described in breeds of lean pork production, penalizes BIW and BIW homogeneity of the piglets. On turns, these effects penalize postnatal development, within-feedlot homogeneity, time for reaching target-weight and carcass and meat quality. These effects were also influenced by the sex of the newborn.

*Effects of litter size on birth-weight and homogeneity*

A higher litter size (LS) was undeniably related, within the litter, to lower mean BIW, increased BIWV and a higher incidence of LBIW and VLBIW piglets. Such effects have been amply described for lean breeds (Milligan *et al.* 2002; Foxcroft *et al.* 2006; Quesnel *et al.* 2008), and these last authors addressed that, besides LS, BIWV is also affected by genotype, parity and management and nutritional (epigenetic) factors. Our results support that LS exerts more striking effects on genotypes with lower prolificacy since, in the current study, the decrease of BIW with increases in LS was higher (43 g of BIW per additional piglet born) than in lean genotypes (33-35 g of BIW; (Quesnel *et al.* 2008; Beaulieu *et al.* 2010). Concomitantly, significant increases in the appearance of VLBIW piglets is found in larger litters than 16 piglets in hyperprolific lines (Quesnel *et al.* 2008),

while in the Iberian breed was found from 10 piglets onwards; being its prolificacy average between 6 and 7 piglets. In this sense, we have to underline that the commercial farm where the study was developed is a good example of current trends for modern Iberian production systems, where selection pressure has increased the percentage of large litters larger than the breed average LS (almost 50% of the total litters in our study) and therefore the incidence of VLBIW piglets.

*Effects of offspring birth-weight and sex on postnatal development and fatness*

In brief, a lower BIW was related with higher mortality during the suckling phase (as in lean breeds (Quiniou *et al.* 2002; Wolf *et al.* 2008)), and significant differences in postnatal development patterns (as also described for lean breeds; (Quiniou *et al.* 2002; Wu *et al.* 2006; Beaulieu *et al.* 2010). Overall, mean body weight and ADWG were highly influenced by BIW during suckling and transition phases. In this way, VLBIW piglets showed lower values than LBIW and LBIW had lower values than MBIW and HBIW offspring. Conversely, in spite that differences in body-weight between MBIW and HBIW piglets continued during these phases, the ADWG was similar in both periods in both groups.

We have to highlight that LBIW, but not VLBIW, increased ADWG when compared to heavier littermates during the period between weaning and end of the transition phase, while both groups increased ADWG at 110 days-old and the weight of all BIW categories were more equaled; these effects were more evident in females and were lost at next growing periods and the fattening phase. This development could be confirmed by FCR, especially, in VLBIW females, which did not maintain the expected growth rhythm during the growing or fattening phases and this increased their DaM. Such data support the existence of a catch-growth effect during the transition period and the beginning of the growing phase (mainly evidenced in the female sex) for increasing the survival possibilities of the lighter piglets (Gonzalez-Bulnes *et al.* 2012a; Ovilo *et al.* 2014). Previous studies of our group (Ayuso *et al.* 2015a; Ayuso *et al.* 2016) have related this catch-up growth with changes in gene expression and gene pathways involved in cell growth and proliferation or protein turnover, which underlines the resilience of traditional breeds like the Iberian pigs.

It is also important to note that the mean body weights at the end of the transition phase and at the different periods assessed during the growing-fattening phases were strongly related to body weight at weaning, even more than BIW, which indicates that any process compromising adequate development during the suckling phase will

compromise later development. Conversely, a higher BIW was related to higher overall ADGW during the study period and, consequently, an earlier DaM, regardless of scarce differences between MBIW and HBIW. Hence, VLBIW females, despite catch-up growth at early postnatal stages, showed the lowest market weight and therefore the longest DaM value.

The differential effects of BIW and sex on postnatal development were also observed when assessing backfat deposition. The pigs of the VLBIW category showed lower fat deposition than the other categories in the earlier life-periods, but both VLBIW and LBIW pigs showed higher backfat depth at older ages (excepting VLBIW females). These results agree with data from commercial breeds (Gondret *et al.* 2005a; Attig *et al.* 2008; Schinckel *et al.* 2010) and support that lighter pigs have a higher trend for accumulating fat, in accordance with data supporting the hypothesis of prenatal programming. The assessment of inner and outer layers separately evidenced no significant effects of BIW on the outer layer depth, but the inner layer, which has more metabolic activity (Hausman and Thomas 1984), was larger in LBIW than in MBIW and HBIW pigs.

However, there were not found major differences when assessing indexes of lipid metabolism through the different developmental stages of the study. Conversely, there were differences in glucose metabolism related to BIW at the end of the study. The category of VLBIW pigs showed higher glucose concentrations than the other groups, which may be linked to an early prodrome of insulin resistance, modulated by sex since VLBIW females had a low secretion of insulin but VLBIW males had the highest secretion of insulin in all the categories of BIW and sex.

Backfat depth was also affected by sex since males showed a significant thicker total backfat than females at weaning and at 215 days-old. Conversely, differences were not significant at 110 days-old, which may be related to a higher depth of the outer layer in females at such age, which in turn may be related to the higher ADWG of females at this age.

Overall, these results indicate that individuals with lower BIW do not compensate their low BIW during postnatal growth and are less efficient than heavier littermates, similarly to previous findings in lean breeds, addressing that (Gondret *et al.* 2005b; Rehfeldt and Kuhn 2006; Bérard *et al.* 2008; Beaulieu *et al.* 2010). Therefore, decreases in mean BIW of the litter and increases in the incidence of VLBIW and LBIW piglets enlarge the DaM period and, at the same time, raise production costs. According to our results,

each 100 g of reduction in BIW implies around 3 more days to reach DaM. In a lean genotype, Beaulieu *et al.* (2010) found 10 days of difference for reaching DaM between the lightest and heaviest BIW groups. In our result, the difference was larger, being 15 days for males and 43 days for females. Thus, fatty breeds showed a much higher influence of BIW on DaM than lean breeds.

*Effect of offspring birth-weight and sex on carcass and meat quality at slaughter*

The results regarding carcass traits showed shorter carcass length for VLBIW pigs than for the other pigs, which should be linked to a worse development in the growing-fattening phase. In fact, VLBIW females had the lowest carcass length and weight which is consistent with their poorer postnatal development. Such effect is well-known in lean breeds, with carcasses from lighter BIW pigs having a lower weight of primary cuts, lower meat content and poorer meat quality (Rekiel *et al.* 2014).

In spite of findings in previous ages, there were not differences of backfat depth between BIW and sexes categories at slaughter. These data are in agreement with previous studies in Iberian pigs (Daza *et al.* 2016; Egea *et al.* 2016; Martinez-Macipe *et al.* 2016). Conversely, IMF content was larger in LBIW piglets than in the heaviest BIW groups and in males than in females; which it is also in agreement with earlier studies in both lean and fatty breeds (Rehfeldt *et al.* 2008; Egea *et al.* 2016; Martinez-Macipe *et al.* 2016). Possible causes for IMF deposition in LBIW pigs may be related to hyperplasia process (increases in adipose cell number) during prenatal stages (Hausman *et al.* 2014). Moreover, the liver fat content was also higher in males than in females with VLBIW females showing the lowest content. This could be related to the altered pattern of growth during the growing and fattening phases.

Fatty acids in tissues are mostly distributed in PL and NL fractions, being predominant the NL fraction (Wood *et al.* 2008). In the IMF, this fraction might be used as an estimator because it represents 70% of total FA (Ayuso *et al.* 2015b). Moreover, the membrane composition (PL) is more stable in its composition than the storage lipids (NL) because of its functional properties (Sampels *et al.* 2011).

The loin is one of the most important carcass cuts in pork production and has a high economic value in Iberian pig. Alvarenga *et al.* (2014) did not detect any effect of BIW on IMF composition comparing light and heavy BIW pigs, but in our study, there were significant differences in the NL fraction of IMF between these groups. Sampels *et al.* (2011)

suggested that there are different mechanisms for the metabolism of different FA in different tissues. This is in line with our results because the effects of BIW categories differ between liver and IMF. In the IMF NL fraction, main differences are between MBIW and HBIW pigs. The HBIW group showed greater C18:1n-9, MUFA, desaturation indexes and UI than the MBIW group. In Iberian pig, the C18:1n-9 has been used as an indicator of meat quality products and high levels in backfat or meat pieces are linked to relevant traits of meat product quality, highly valued by consumers, because of the association with the production of oleic acidays-derived volatiles (Barea *et al.* 2013). Moreover, increase in PUFA and MUFA meat levels is a priority because it would improve consumers' acceptance due to possible health and sensorial benefits (Laitinen *et al.* 2006; Jakobsen *et al.* 2009). In the PL fraction of IMF, LBIW, MBIW and HBIW groups also showed greater PUFA values and UI, than VLBIW group. The BIW groups with the highest values were MBIW and HBIW groups. In the SCF, the most important difference was the greater n-3 FA in HBIW pigs than in MBIW pigs, due to higher C18:3n-3. Moreover, the lowest values of these variables were obtained in the VLBIW group and this is less interested in meat quality.

As liver is a main viscera related to metabolism, the metabolism status of individuals might affect physiological processes. The differences between BIW categories in liver showed a higher MUFA and desaturation indexes in the LBIW group than in the other groups. The SCD1 activity has been related to metabolic disorders such as obesity or insulin resistance (Hulver *et al.* 2005; Poudyal and Brown 2011), although the LBIW showed a regular value of insulin at market. However, this higher activity of SCD1, which plays a central role in *de novo* lipogenesis, might be linked to the beginning of fat storage liver increase. This event is supported by the higher C16:1 content of LBIW pigs, because this FA is considered a marker of adiposity (Paillard *et al.* 2008). Other studies have found that a decreased SCD1 activity promotes lipid oxidation in lipid storage (Dobrzyn *et al.* 2004; Dobrzyn *et al.* 2005). This lower activity could be related to the lowest ratios of desaturation indexes in VLBIW females and the lowest liver fat content. Moreover, SCD1 deficiency might lead to increase of SFA and VLBIW females had the greatest SFA concentration. Regarding the differences between the LBIW group and the heaviest BIW groups, the higher values of C20:5-n3 and C22:6-n3 (which derive from the essential FA C18:3-n3) in the heaviest BIW groups stand out because of the beneficial effects of n-3 FA. These FA are negative regulators of hepatic lipogenesis (these pigs showed less SCD1 activity than LBIW pigs) and inflammatory response and they are considered beneficial for some metabolic disorders (Simopoulos 2010). Further experiments about protein levels

and gene expression of lipogenesis and lipolysis enzymes could be interesting for a deeper understanding of fat metabolic processes in LBIW pigs.

The sex-related effect on FA profile has been reported in many studies (Segura *et al.* 2015a; Daza *et al.* 2016; Egea *et al.* 2016). Males and females showed differences in SCF, IMF and the PL fraction of liver with a quite homogeneous pattern. Overall, males had greater MUFA, unsaturation indexes and monounsaturated C18, but lower SFA than females. Moreover, males showed more C18:3-n3 than females in the PL fractions and the inner layer of SCF. Moreover, males had a lower ∑n−6/∑n−3 ratio in the outer layer and greater UI in both layers of SCF than females. This data is interesting because of the human diet recommendations of a ∑n−6/∑n−3 ratio of 1/4 and higher MUFA and PUFA values, as described above (Simopoulos 2002). This FA profile of this study is clearly better for males than for females regarding meat quality because, as described above, more n-3 FA, MUFA and C18:1-n9 are desirable characteristics for IMF and SCF.

## Conclusions

The present study clearly supports, for fatty breeds, that strong increases in LS are related to higher within-litter BIWV and higher incidence of lighter BIW piglets. Our results also support the adverse effects of low BIW on postnatal growth traits and meat quality in fatty pigs. Piglets with lower BIW, despite processes of catch-up growth, have lower ADWG and higher FCR during the phases of growing and fattening, which increases daily feeding costs and induces a longer period for achieving weight-target. Remarkably, VLBIW females and males took 43 and 15 days longer respectively than HBIW females and males to reach the market. This situation, of course, increases production cost and diminish benefits.

Regarding carcass and meat traits, lighter BIW piglets have worse quality. Moreover, the HBIW group showed higher meat quality than MBIW pigs in the FA profile of IMF. All these effects were stressed by sex since females showed less growth potential than males during the growing phase and, at the market, produced meat with worse sensorial and health quality attributes than males.

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## Conflicts of Interest

The authors declare no conflicts of interest

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# 4.2. Experimento 2

## Maternal undernutrition and offspring sex determine birth-weight, postnatal development and meat characteristics in traditional swine breeds

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Consultar el material suplementario correspondiente en el Anexo 3

## Maternal undernutrition and offspring sex determine birth-weight, postnatal development and meat characteristics in traditional swine breeds

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## Abstract

### Background

The aim of this study was to determine how maternal undernutrition during pregnancy and offspring birth-weight can affect the postnatal development of offspring under farm conditions, which may lead to consequences in its meat and carcass quality. The current study involved a total of 80 litters from Iberian sows fed a diet fulfilling daily requirements (n=47; control) or providing 70% daily requirements (n=33; underfed) from day 38 to day 90 of gestation when fetal tissue development begins. After birth, piglets born live were classified as low birth-weight (LBW; < 1kg) and normal birth-weight (NBW, ≥1kg). During the growing phase, 240 control and 230 underfed pigs (50% males and females) distributed by BW category and sex were studied until the slaughter.

### Results

At birth and weaning, there were significant differences in all morphological measures and weight between NBW and LBW piglets as expected ($P$<0.0005), but few effects of the gestational feed restriction. During the growing phase, NBW pigs continued with higher weight than LBW pigs on all the days of evaluation ($P$<0.05), even though control-LBW-females and LBW-males showed a catch-up growth. However, underfed pigs showed slower growth and higher feed conversion ratio than control pigs ($P$<0.0001) at 215 days old. Moreover, the average daily weight gain (ADWG) for the overall period was greater for NBW, male and control pigs than for their LBW, female and underfed pigs ($P$<0.0001, <0.0005 and <0.05, respectively) and NBW pigs were slaughtered at a younger age than LBW pigs ($P$<0.0001). After slaughtering, control pigs also had higher carcass yield and backfat depth than underfed pigs ($P$<0.0005) and the maternal nutritional effect caused main changes in the polar lipid fraction of liver and loin. The fatty acid composition of loin in control pigs had higher C18:1n−9 and n-3 FA concentrations, as well as lower $\sum$n−6/$\sum$n−3 ratio, than in underfed pigs ($P$<0.005).

### Conclusions

In brief, results showed that the effects of maternal nutritional restriction appeared and increased with offspring age, causing worse developmental patterns for underfed pigs than for control pigs.

**Keywords:** Carcass quality, Fatty acids, Feed restriction, Growth, Low birth-weight, Iberian pigs, Malnutrition.

## Background

Modern swine production (mainly located in Europe, USA, Brazil, China and other countries from Southeast Asia) is based on highly-selected genotypes (mostly originated from Landrace, Large-White and Pietrain breeds) reared in large farms generating value-for-money fresh pork products. In addition to this intensive production, there is also a local European industry (currently spreading to some countries of Asia and America) which is based on the use of traditional breeds for the elaboration of high-quality dry-cured products (ham, cured-loin, spiced-sausage, and salami). The most representative traditional breeds are the Iberian (Spain and Portugal) and Mangalica (Hungary) pigs, but there are other breeds reared in France, Germany, and Italy. These pigs have a high potential for fat accumulation and the content and composition of intramuscular fat give pork products their smooth texture and outstanding taste. In practice, these individuals are reared either as purebred or crossbred with Duroc boars for improving meat yields. Currently, the increasing demand for dry-cured ham and other gourmet products is transforming the traditional extensive farming of these pigs into more intensive systems, with management practices directly implemented from modern breeds; mainly at the reproductive (aiming to increase the number of piglets/litter) and nutritional levels (imitating feeding strategies).

The number of piglets per litter (prolificacy) is determined by the number of ovulations and, afterward, by the number of developing conceptuses. However, studies in modern breeds have shown that higher prolificacy compromises the proper fetal development, due to competition among littermates for the limited space available in the uterus for implantation and adequate placental development [1]; the so-called uterine capacity. Inadequacies of placental development affect the functionality of the organ and, therefore, the supply of oxygen and nutrients to the fetus. The consequence is the impairment of fetal growth (a process known as intrauterine growth restriction; IUGR), leading to low birth-weight (LBW) neonates [2]. The greater number of piglets in the litter, the higher incidence of LBW individuals [3, 4]. Traditional breeds like Mangalica and Iberian pig are characterized by a lower prolificacy and a smaller uterus (a lower uterine capacity) than prolific sows [5, 6]. Therefore, small increases in prolificacy are directly related to

increases in fetal losses and LBW incidence [7]. The implementation of feeding strategies based on maternal feed restriction for lowering production costs in modern breeds has also been found to be related to a higher rate of IUGR processes and LBW piglets [2, 8]. The same has been found in the Iberian pig [9, 10].

The pork market, both in modern and traditional systems, requires batches of pigs with uniform weight, carcass conformation, and meat composition; even more in the case of high-quality products. Hence, LBW pigs reduce the value of farm products. Firstly, the appearance of LBW pigs causes a lack of homogeneity within litters and feedlots. Second, LBW piglets show higher morbidity and mortality, and lower growth potential, lower feed efficiency and lower meat yield than their normal birth-weight (NBW) littermates [11-13]. Moreover, LBW piglets may modify their physiology and metabolism by prenatal programming in response to the inadequate intrauterine environment, either of maternal or placental origin [14, 15]. After birth, these individuals are predisposed to excess adiposity as an adaptive mechanism for energy storing and survival in the inadequate postnatal environment expected, so carcass yields and meat quality would be affected.

In summary, these differences cause variability in carcass conformation and meat quality among pigs in the same litter and feedlot, which is negative for commercialization of the products. Such effects may be even more evident and negative in traditional swine. Firstly, due to the intrinsic developmental patterns of the breeds, in which IUGR individuals are even more prone to fat deposition after prenatal restriction [16-18]. Moreover, Iberian sows are highly predisposed to the mobilization of fat depots, insulin resistance and dyslipidemia in the case of undernutrition and maternal dyslipidemia affects placental efficiency and fetus metabolism and growth [7], increasing incidence and consequences of LBW offspring. Finally, traditional systems are based on longer productive cycles, where animals are slaughtered at a higher weight and therefore older age than lean commercial breeds (140-160 kg live-weight at 10-12 months old). Consequently, homogeneity of feedlots is affected due to over-time increased growth differences between LBW and NBW pigs with differences of several weeks or even months for reaching the target weight for the slaughterhouse.

Despite these considerations, there are scarce data regarding incidence and consequences on postnatal development and meat characteristics of LBW offspring in Iberian pigs and similar breeds reared in commercial farms. Hence, the aim of this study was to determine, for Iberian pigs under farm conditions, the effects of birth-weight and

strategies based on maternal feed restriction on at-birth phenotypic characteristics, postnatal development and metabolism, and meat and carcass quality.

## Material and Methods

### Animals and handling

Animal management was performed in agreement with the Spanish Policy for Animal Protection RD53/2013, which meets the European Union Directive 2010/63/UE about the protection of animals used in research. The experimental procedures were assessed and approved (report CEEA 2012/036) by the INIA Committee of Ethics in Animal Research (the Institutional Animal Care and Use Committee). All the animals (sows and piglets) were housed indoors, with a controlled temperature of around 22 °C, at the farm of Ibéricos de Arauzo 2004 S.L. (Zorita de la Frontera, Salamanca), either at individual (sows) or collective pens (offspring).

The design of the study is shown in Fig. 1. Each of the steps of the procedure is detailed in this section. The study involved a total of 80 litters from Iberian sows inseminated with Duroc cooled semen (PIC, Genus plc, UK). The sows were of 3rd and 4th parity and were evenly divided into two experimental groups. Forty-seven sows (CONTROL group) were individually fed with a standard grain-based diet by chip identification throughout the entire pregnancy (composition at Supplementary Table 1, Additional File 2), calculated for fulfilling daily maintenance requirements for Iberian breed [19]. Thirty-three sows (UNDERFED group) were fed the same diet, but the amount was adjusted to fulfill only 70% of their daily maintenance requirements from days 38 to 90 of pregnancy. At farrowing, the numbers of total piglets born and born live were determined for each sow and, immediately, all the live piglets were examined for sex determination, weight recording and individual identification with earrings. Piglets were classified by their birth-weight (BW) as low and normal BW (LBW and NBW, respectively). The criterion for LBW was defined as a BW lesser than one standard deviation of the mean value of the control littermates (BW<1kg [20, 21]). Piglets remained with the mothers until weaning, after within-treatment fostering for equaling the number of piglets among sows. Offspring were managed following the regular practices of the farm.

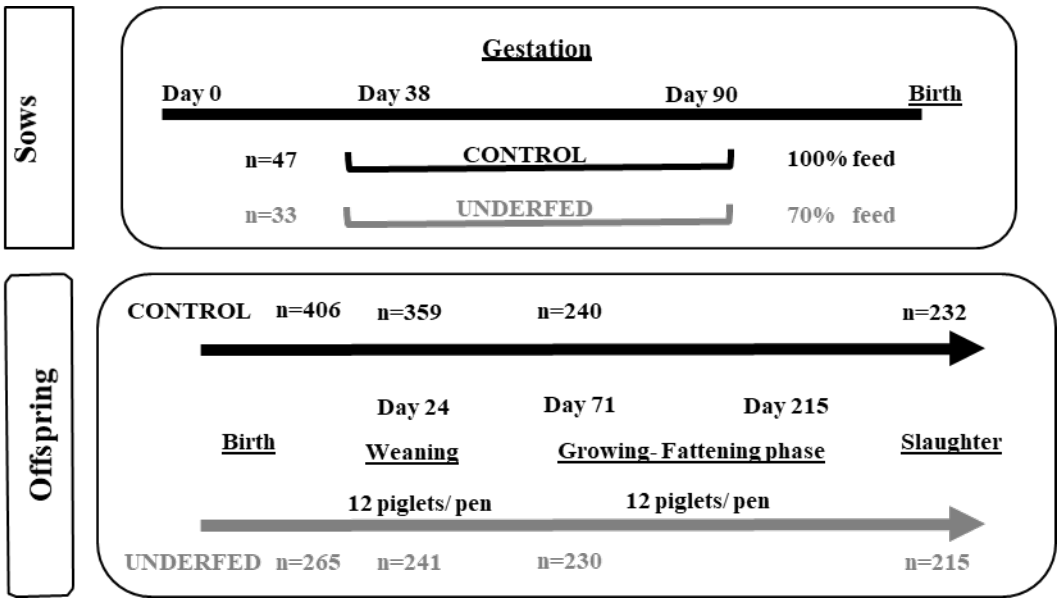


**Fig. 1:** Schematic representation of study design.

The study involved 406 and 265 piglets born live from control and underfed pregnancies, respectively, with similar percentages of females and males (overall: 49.2% females vs. 50.8% males). At weaning, performed around 24 days of age, piglets were grouped by maternal diet, BW, and sex in pens of 12 piglets. Postnatal development was assessed during the transition phase (25 to 70 days of age) in a total of 359 control (20 LBW and 166 NBW females, and 21 LBW and 152 NBW males) and 241 underfed piglets (14 LBW and 106 NBW females, and. 15 LBW and 106 NBW males). Afterward, 240 control (16 LBW and 104 NBW pigs by each sex) and 230 underfed (12 LBW and 103 NBW pigs by each sex) pigs were randomly selected for studying development during the growing and fattening phases (from 71 days old to the slaughter). Finally, a total of 232 control (115 females and 117 males) and 215 (107 females and 108 males) underfed pigs were slaughtered after reaching the minimum weight established by the Spanish Policy for Iberian Products (115 kg of carcass weight).

### Assessment of postnatal development and yield indexes

At birth and weaning, all the piglets were weighed and measured for occipito-nasal length, biparietal diameter, trunk length, maximum thoracic diameter and abdominal and thoracic circumferences. Animals were weighed again at average ages of 110, 150, 180 and 215 days, and final slaughter-weight and age were recorded at the slaughterhouse. These values were used for calculating average daily weight gain (ADWG) and feed conversion ratio (FCR) in four intermediate periods of age: 25-110, 111-150, 151-180 and 181-215 days of age (each period is named as its last day). The ADWG values for suckling phase and whole

life were also calculated. The formula of ADWG was [(final weight-initial weight)/number of days], while FCR was determined as [daily block feed intake mean/ADWG of the period]. Furthermore, backfat depth (total and divided in outer and inner layers) and loin diameter were determined at the P2 point at the level of the head of the last rib, at weaning and at 215 days old by using a SonoSite S-Series ultrasound machine equipped with a multifrequency lineal array probe (5-8 MHz; SonoSite Inc., USA).

**Assessment of carcass features and tissue sampling at slaughter**

Total weight, total length and backfat thickness were recorded for all the carcasses immediately after slaughter. Carcass length was determined from the posterior edge of the symphysis pubica to the anterior edge of the first rib, while backfat thickness was measured in the midline of the carcass and at the level of the last rib (skin not included). In each pig, the value of carcass weight was used to determine carcass yield by using the formula [carcass weight/body weight], expressed as a percentage.

Immediately after slaughter, samples of subcutaneous fat and longissimus dorsi (LD) muscle were drawn at the midline of the carcass and collected from the level of the last rib (at the point used for measuring backfat depth) and samples of hepatic tissue were obtained from the right lobe of the liver. All these samples were immediately packaged in individual bags and stored at -20°C until analyzed for fatty acids (FA) composition analysis. A second sample of LD was used for analyzing drip-loss and moisture. In brief, at the same day of sampling, drip loss of LD muscle was determined by using the method described by Calvo et al. [22], while moisture was determined by drying samples at 110°C [23].

**Evaluation of plasma indexes of carbohydrates and lipids metabolism**

At 215 days old, a blood sample for each pig was drawn from the orbital sinus by using 5 ml sterile heparin vacuum tubes (Vacutainer Systems Europe, France). At slaughter, individual blood samples were collected with 5 ml sterile heparin tubes. Immediately after recovery, blood samples were centrifuged at 1,500xg for 15 min and the plasma was separated and biobanked into polypropylene vials at -20 °C until the analysis of metabolic biomarkers (glycemic values and lipids profile). Glucose and fructosamine (parameters related to glucose profile), as well as parameters related to lipid profile total cholesterol, high-density lipoprotein cholesterol (HDL-c), low-density lipoprotein cholesterol (LDL-c) and triglycerides, were measured in plasma. Fructosamine is considered a better index

than glucose itself for long periods since it represents average glucose values during previous days [24]. Assays were performed with a clinical chemistry analyzer (Saturno 300 plus, Crony Instruments s.r.l., Rome, Italy), according to the manufacturer's instructions. The data of kits (SPINREACT, Sant Esteve de Bas, Spain) and reliability criteria for all assays were included in Additional File 1.

**Evaluation of fatty acid composition of diets**

Dietary FA were extracted and methylated by the one-step procedure of Sukhija et al. [25]. Fatty acid methyl esters (FAME) were analyzed and identified by gas chromatography (Hewlett Packard HP-6890, USA) with a flame ionization detector and a capillary column (HP-Innowax, 30 m × 0.32 mm i.d. and 0.25 μm polyethylene glycol-film thickness) with a temperature program of 170 to 245 °C as previously described [26]. Results were expressed as gram per 100 g of detected FAME.

**Evaluation of the fat content and fatty acid composition of tissue samples**

The procedure of fat extraction was different for backfat samples and liver and muscle samples. In brief, subcutaneous fat was extracted after being separated in outer and inner layers. Each layer was studied separately because the inner layer is metabolically more active than the outer layer, mainly due to a high lipoprotein lipase activity [27]. In the case of liver and LD muscle, samples were previously freeze-dried for three days in a lyophilizer (Lyoquest, Spain) and ground in a Mixer Mill MM400 (Retsch Technology, Germany) until tissues were completely powdered. Liver and LD lipids were extracted as described by Segura et al. [28] and fat tissue was calculated and expressed as a percentage. Afterward, lipids of liver and LD were separated in neutral and polar lipids (NL and PL, respectively) by using aminopropyl minicolumns accordingly with the method employed by Ruiz et al. [29] methylated with pentadecanoic acid (C15:0; Sigma, Spain) as the internal standard [30] and analyzed by gas chromatography as described by Lopez-Bote et al. [26]. The percentages of individual fatty acids were used to calculate proportions of saturated (SFA), monounsaturated (MUFA) and polyunsaturated (PUFA) fatty acids, as well as total n-3 and n-6 and their ratio (Σn-6/Σn-3). The activity of stearoyl-CoA desaturase enzyme 1 (SCD1) was estimated as C18:1/C18:0, while ratios for MUFA/SFA and unsaturation were determined [31, 32].

**Statistical analysis**

Data were analyzed using the general linear model (GLM) procedure contained in the SAS version 9.4 (Statistical Analysis System Institute Inc., USA). The model included nutritional status of the sow (control/underfed), BW classification (LBW/NBW) and sex (female/male) as the main effects. Interactions of two-way (BW classification x Nutritional status and BW classification x sex) and the three-way interaction of main effects were examined and showed in tables. Statistical analysis of birth and weaning were blocking for sow to account for the common maternal environment. Litter size (LS), which was categorized into three groups (3 to 6 piglets/litter, 7-9 piglets/litter, and 10-13 piglets/litter), was used as a random effect for birth data. For performance parameters, age was used as a covariate. Moreover, Duncan's test was used to identify differences between groups. Chi-square was used to assess the mortality data and the percentage of births by the litter size classified into three groups, as described above. The piglet was the experimental unit for all the variables studied except for the reproductive parameters where sow was the unit, and for the FCR data with the pen as experimental unit. All the results were expressed as mean ± RMSE (root mean square error) in tables, but mean ± SD (standard deviation) was used for reproductive parameters, figures and text. Statistical significance was accepted from $P<0.05$ and trend was defined between P-values of 0.05 and 0.10.

## Results

### Effects of maternal undernutrition on prolificacy and litter size

At farrowing, there were not significant differences between control and underfed groups in the total number of piglets born/litter (8.9±2.6 vs. 8.5±2.0 for control and underfed sows, respectively) or piglets born live/litter (8.6±2.6 vs. 8.0±1.8 piglets for control and underfed sows, respectively). Concomitantly, at weaning, there were no significant differences in the mean total number of weaned piglets/litter (7.7±0.7 vs. 7.4±0.7 piglets for control and underfed sows, respectively) or in the total litter-weight/litter (42.1±7.6 kg vs. 41±7.5 kg for control and underfed sows, respectively).

Despite the absence of significant differences in the mean number of piglets/litter, the analysis of LS showed a similar percentage of litters with 3-6 piglets in control and underfed sows (17 vs. 15.2%, respectively) but suggestive differences in the distribution of litters with more than seven piglets. Control sows showed 34.0% of medium litters with 7-

9 piglets and 48.9% of large litters with 10-13 piglets, while underfed sows had 54.6 and 30.3% for medium and large, respectively. There were trends for a lower percentage of litters with 7-9 piglets (P=0.07) and a higher percentage of litters with 10-13 piglets (P=0.09) in the control group than in the underfed group.

**Effects of maternal undernutrition and offspring sex on characteristics of piglets at birth**

The nutritional status of the sow during pregnancy did not have significant effects on birth weight and most of the body measures of piglets, except control piglets showing larger occipito-nasal length, shorter trunk length and higher thoracic diameter than underfed counterparts (Supplementary Table 2, Additional File 3). The incidence of LBW piglets was directly related to LS in both groups (P<0.001), being higher in control than in underfed sows (15.8% control vs. 8.4% underfed; P<0.005). The distribution of this incidence was 1.5% in litters of low LS (2.4 vs. 0% for control and underfed litters, respectively), 7.8% in medium litters (5.7 vs. 9.7%, respectively) and 19% in litters with more than nine piglets (23.2 vs. 8.8%, respectively). As expected, weight was higher in NBW than in LBW piglets at birth (1.4±0.2 vs. 0.8±0.2 kg, respectively) and weaning (5.6±1.2 vs. 4.3±1.1 kg; P<0.0001 for both). Occipito-nasal and trunk lengths and abdominal and thoracic circumferences were also greater at birth and weaning in NBW (birth: 12.6±0.6, 23.5±1.8, 19.0±1.6 and 24.3±1.5 cm and weaning: 16.2±0.9, 40.1±3.8, 33.4±3.7 and 38.5±3.3 cm, respectively) than in LBW piglets (birth: 11.7±0.6, 19.6±1.6, 15.4±1.6 and 19.8±1.9 cm and weaning: 15.3±1.1, 35.6±4.1, 30.5±3.1 and 35.0±3.6 cm, respectively; P<0.0005 for all). There were no significant effects of sex on the total incidence of LBW piglets, being 14.5% for males and 11% for females. On the other hand, there were sex-related effects on the thoracic circumference at birth; such value was greater in females than in males (23.9±2.0 vs. 23.5±2.3 cm, respectively; P<0.01) and LBW-males showed the smallest circumference (19.4±2.0 cm; P<0.05 for the interaction effect).

**Assessment of early-postnatal development (suckling phase)**

The analysis of ADWG during lactation and weight at weaning showed an interaction between maternal diet and BW (Fig. 2 and Supplementary Table 2, Additional File 3). The NBW piglets of the control group showed higher ADWG and weight than the NBW piglets from underfed sows. The effect was the opposite in the LBW piglets since LBW piglets of the underfed group had higher ADWG and weight than LBW piglets of the control group (Fig. 2; P<0.005 and P<0.05 for the interaction effect, respectively). These effects were also found when assessing the backfat depth, which was higher in underfed-LBW and control-

NBW groups than in the remaining piglets (Fig. 2; P<0.05 for the interaction effect). Conversely, such interaction was not found in the values for loin diameter, and NBW and control piglets (0.9±0.3 and 1.0±0.2 cm) had higher values than LBW and underfed piglets (0.8±0.3 and 0.8±0.2 cm) respectively (P<0.0001 for birth weight and P<0.01 for maternal diet). The assessment of body size showed the largest occipito-nasal and trunk lengths and abdominal and thoracic circumferences in the control-NBW piglets (16.3±1.0, 40.2±4.1, 34.4±3.4 and 38.9±3.5 cm, respectively) and the smallest in the control-LBW piglets (15.2±1.2, 35.0±4.5, 30.4±3.4 and 34.7±3.9 cm, respectively; P<0.05 for the interaction effect). Moreover, the abdominal perimeter was greater in the control group than in the underfed group (33.9±4.0 vs. 31.9±2.8 cm, respectively; P<0.05).

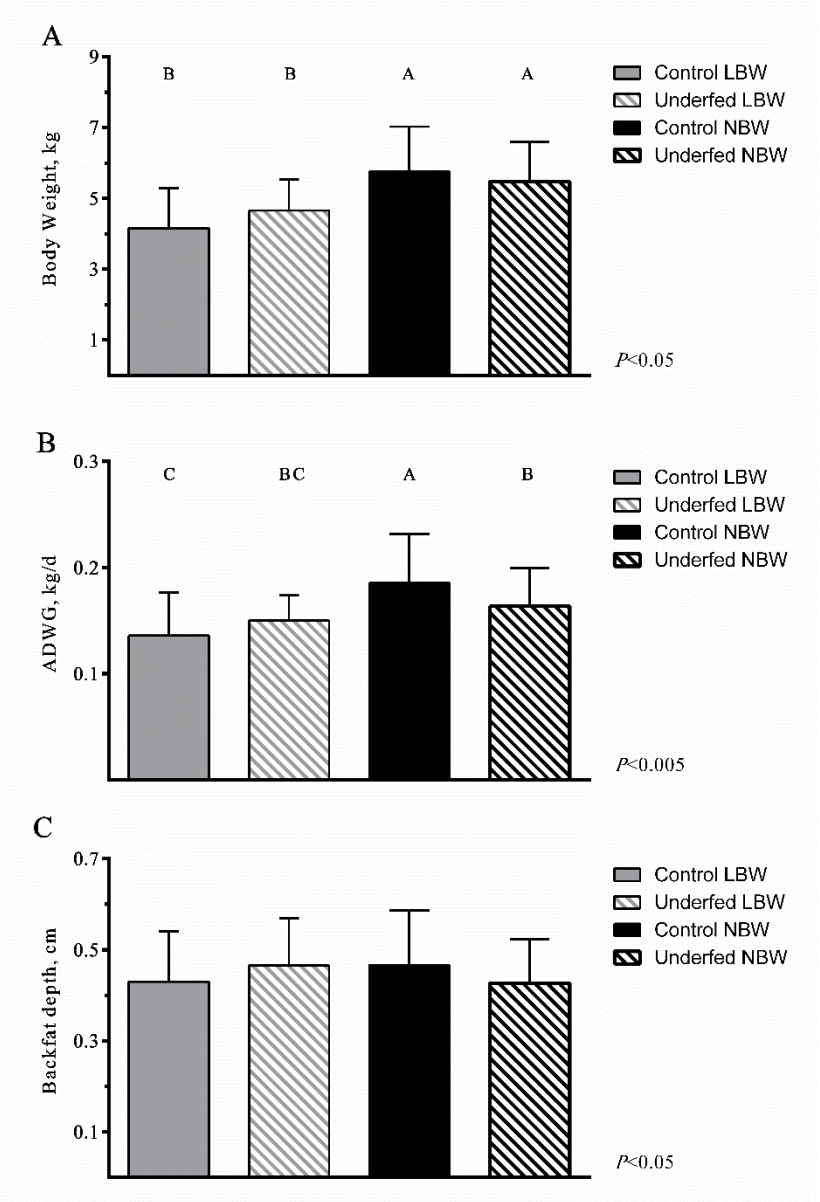


**Fig. 2:** Effect of birth-weight and nutritional status of sows at weaning. Mean ± SD values of body weight (Panel A), ADWG (Panel B) and backfat depth (Panel C) in control LBW,

underfed LBW, control NBW and underfed NBW pigs at 24 days old. Different letters indicate significant differences by Duncan's test. LBW= Low birth weight, NBW= Normal birth weight, ADWG= Average daily weight gain.

**Assessment of late-postnatal development (growing-fattening phase)**

It is noteworthy that, during the growing-fattening phase, mortality was higher in the underfed group than in the control group (6.5% control group vs. 3.3% control group; $P<0.05$), especially in the period from 215 days old to slaughter.

The maternal diet also had a significant influence on body weight, ADWG and FCR. At 110 days old, underfed-LBW-females showed the lowest body weight and ADWG values and the greatest FCR (Supplementary Table 3, Additional File 4; $P<0.005$ for the interaction effects) while control-LBW-females had the lowest FCR and the greatest ADWG. However, control-LBW-females showed the highest FCR and the lowest ADWG at 150 days old ($P<0.05$ for the interaction effects). Afterward, body weight was lower at 180 days old in offspring from controls sows (82.3±11.5 vs. 84.7±12.4 kg; $P<0.05$), but higher at 215 days old than in offspring from underfed sows (113.6±13.8 vs. 107.2±14.3 kg; $P<0.0003$; Fig. 3). Furthermore, underfed pigs had also lower ADWG and higher FCR than control pigs at 215 days old (0.7±0.1 vs. 0.9±0.1 kg/d and 5.2±1.3 vs. 3.9±0.9 kg/kg; $P<0.0001$ for both).

Birth-weight also significantly affected values of ADWG, FCR, and body weight. In both control and underfed groups, the values for body weight were always higher in NBW than in LBW pigs (150d: 57.7±9.3 vs. 54.7±11.1, 180d: 84±11.5 vs. 78.5±15.1 and 215d: 111.2±13.9 vs. 103.9±17.4 kg; $P<0.05$ for all age-periods; Supplementary Table 3, Additional File 4). From 150 days old onwards, the LBW group showed lower ADWG (150d: 0.54±0.2 vs. 0.6±0.1, 180d: 0.66±0.2 vs. 0.71±0.1 and 215d: 0.75±0.2 vs. 0.82±0.2 kg/d) and higher FCR than the NBW group (150d: 2.4±1.2 vs. 2±1.0, 180d: 4.5±1.4 vs. 3.7±0.7 and 215d: 5±1.6 vs. 4.5±1.2 kg/kg; $P<0.005$ for all).

Offspring sex showed a significant effect from 150 days old; males had higher body weight, higher ADWG and lower FCR than females at 150 (58.4±9.9 vs. 56.5±9.1 kg, 0.62±0.2 vs. 0.58±0.1 kg/d and 2.0±0.9 vs. 2.1±0.9 kg/kg, respectively) and 180 days old (85.7±12.1 vs. 81.2±11.4 kg, 0.8±0.2 vs. 0.7±0.1 kg/d and 3.8±0.8 vs. 3.9±1.0 kg/kg; $P<0.01$, $P<0.0001$ and $P<0.05$ for both periods, respectively). Moreover, LBW-females showed the highest FCR but the lowest ADWG at 180 days old (4.9±1.6 kg/kg and 0.57±0.2 kg/d) and the lowest body weight at 180 and 215 days old (Fig. 3; $P<0.05$ for the interaction effects).

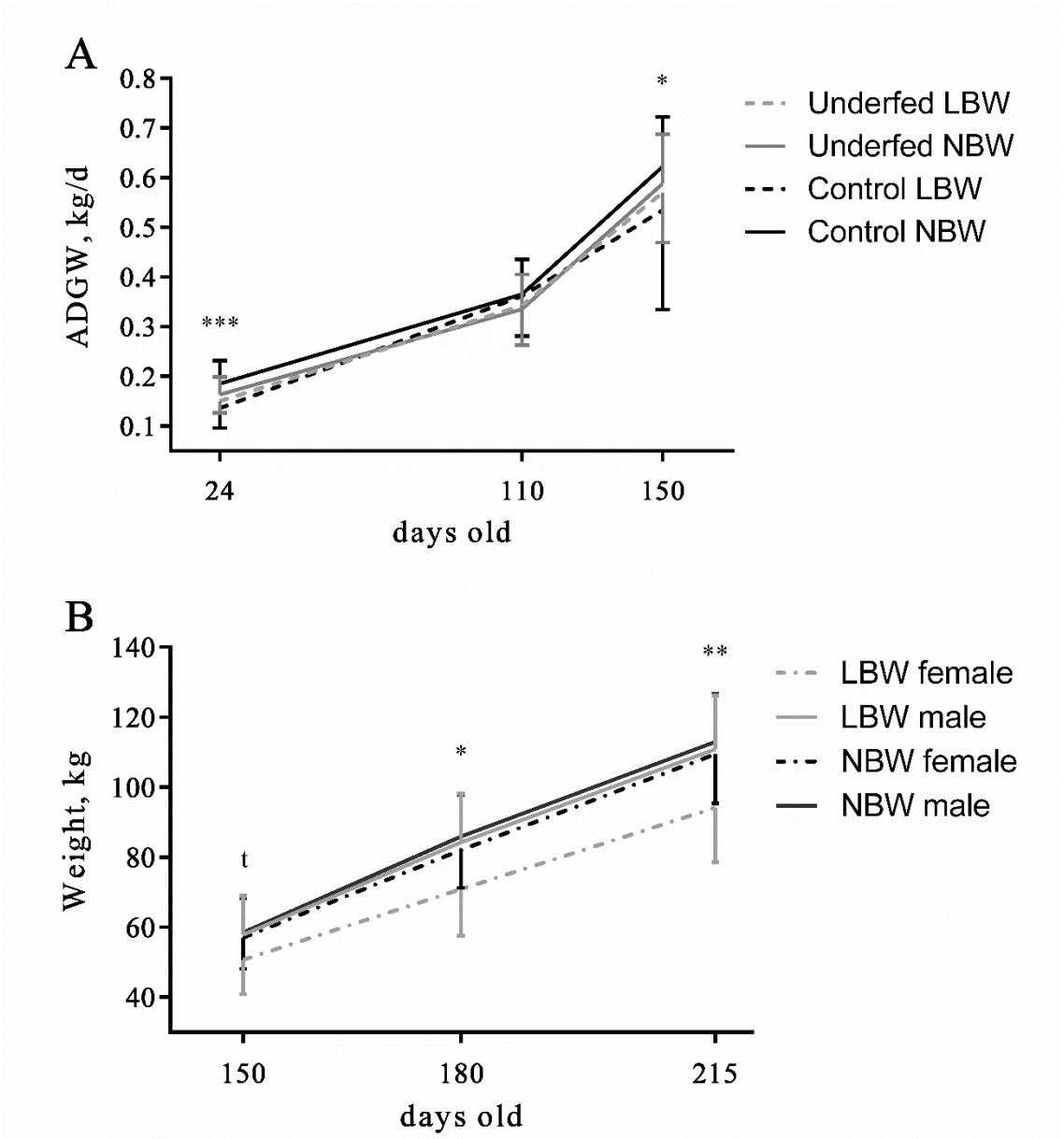


**Fig. 3: ADGW and weight during the growing phase.** Mean ± SD values of ADGW (Panel A) and body weight (Panel B). Panel A: control LBW, underfed LBW, control NBW and underfed NBW pigs. Panel B: LBW female, LBW male, NBW female and NBW male pigs. LBW= Low birth weight, NBW= Normal birth weight, ADWG= Average daily weight gain. Asterisks indicate significant differences between groups (t= 0.1>P>0.05, *=P<0.05, **=P<0.01, ***=P<0.005).

Birth-weight also significantly affected values of ADWG, FCR, and body weight. In both control and underfed groups, the values for body weight were always higher in NBW than in LBW pigs (150d: 57.7±9.3 vs. 54.7±11.1, 180d: 84±11.5 vs. 78.5±15.1 and 215d: 111.2±13.9 vs. 103.9±17.4 kg; *P*<0.05 for all age-periods; Supplementary Table 3, Additional File 4). From

150 days old onwards, the LBW group showed lower ADWG (150d: 0.54±0.2 vs. 0.6±0.1, 180d: 0.66±0.2 vs. 0.71±0.1 and 215d: 0.75±0.2 vs. 0.82±0.2 kg/d) and higher FCR than the NBW group (150d: 2.4±1.2 vs. 2±1.0, 180d: 4.5±1.4 vs. 3.7±0.7 and 215d: 5±1.6 vs. 4.5±1.2 kg/kg; $P<0.005$ for all).

Offspring sex showed a significant effect from 150 days old; males had higher body weight, higher ADWG and lower FCR than females at 150 (58.4±9.9 vs. 56.5±9.1 kg, 0.62±0.2 vs. 0.58±0.1 kg/d and 2.0±0.9 vs. 2.1±0.9 kg/kg, respectively) and 180 days old (85.7±12.1 vs. 81.2±11.4 kg, 0.8±0.2 vs. 0.7±0.1 kg/d and 3.8±0.8 vs. 3.9±1.0 kg/kg; $P<0.01$, $P<0.0001$ and $P<0.05$ for both periods, respectively). Moreover, LBW-females showed the highest FCR but the lowest ADWG at 180 days old (4.9±1.6 kg/kg and 0.57±0.2 kg/d) and the lowest body weight at 180 and 215 days old (Fig. 3; $P<0.05$ for the interaction effects).

At slaughter, the assessment of the ADWG for the overall period showed that control, male and NBW pigs had higher ADWG (0.56±0.1, 0.55±0.1 and 0.55±0.1 kg/d, respectively) than their respective counterparts (underfed, female and LBW pigs, 0.53±0.1, 0.54±0.1 and 0.50±0.1 kg/d, respectively; Table 1). The overall ADWG and slaughter weight were the lowest in LBW-females (0.47±0.1 kg/d and 140.1±18.6 kg; $P<0.005$ for the interaction effects), but they showed the greatest slaughter age (301.6±20.5 days old; $P<0.005$ for the interaction effect). Regarding BW effect, NBW pigs were slaughtered at a younger age than LBW pigs (277.4±19.6 vs. 292.3±21.1 days old, respectively; $P<0.0001$). Moreover, control-NBW and -LBW pigs showed the lowest and the highest age to slaughter (271.9±23.4 and 293.8±25.4 days old, respectively; $P<0.01$ for the interaction effect).

Effects and the interaction of maternal diet, BW and sex were also found when assessing backfat depth; underfed, NBW and male pigs (2.5±0.5, 2.4±0.5 and 2.5±0.5 cm, respectively) had thicker backfat than their respective counterparts at 215 days old (control, LBW and female pigs, 2.3±0.4, 2.2±0.6 and 2.3±0.5 cm respectively; Supplementary Table 3, Additional File 4). Moreover, at such age, males also had a higher loin diameter than females (3.5±0.7 vs. 2.9±0.6; $P<0.0001$).

**Table 1:** Carcass and meat quality traits at slaughter.

| Variables | N | Control | | | | Underfed | | | | RMSE | *P*-value | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | LBW | | NBW | | LBW | | NBW | | | | | | | | |
| | | Females | Males | Females | Males | Females | Males | Females | Males | | BW | Sex | Nutr | BW* Sex | BW* Nutr | BW* Sex* Nutr |
| Body Weight, kg | 442 | 141.5 $^{c}$ | 151.0 $^{a}$ | 153.4 $^{a}$ | 154.0 $^{a}$ | 137.7 $^{c}$ | 147.1 $^{ab}$ | 152.0 $^{a}$ | 151.5 $^{a}$ | 9.7 | <.0001 | 0.004 | t | 0.005 | ns | ns |
| Age, d | 442 | 306.3 $^{a}$ | 284.7 $^{bc}$ | 269.5 $^{d}$ | 274.3 $^{cd}$ | 293.1 $^{b}$ | 287.2 $^{bc}$ | 284.4 $^{bc}$ | 282.2 $^{bcd}$ | 18.9 | <.0001 | ns | t | 0.02 | 0.01 | t |
| ADWG, kg/d | 442 | 0.47 $^{c}$ | 0.54 $^{ab}$ | 0.57 $^{a}$ | 0.57 $^{a}$ | 0.47 $^{c}$ | 0.51 $^{b}$ | 0.54 $^{ab}$ | 0.54 $^{ab}$ | 0.06 | <.0001 | 0.007 | 0.04 | 0.004 | ns | ns |
| Carcass Weight, kg | 395 | 118.0 $^{a b}$ | 121.6 $^{ab}$ | 122.1 $^{a}$ | 122.2 $^{a}$ | 108.0 $^{c}$ | 115.0 $^{b}$ | 118.8 $^{ab}$ | 118.4 $^{ab}$ | 8.1 | 0.002 | ns | 0.0001 | t | ns | ns |
| Carcass yield, % | 395 | 79.96 $^{a b}$ | 80.55 $^{a}$ | 79.26 $^{abc}$ | 79.16 $^{abc}$ | 78.61 $^{c}$ | 78.14 $^{c}$ | 78.13 $^{c}$ | 78.14 $^{c}$ | 1.94 | t | ns | <.0001 | ns | ns | ns |
| Carcass Length, cm | 376 | 88.10 $^{a b}$ | 88.23 $^{ab}$ | 90.55 $^{a}$ | 90.25 $^{ab}$ | 87.83 $^{b}$ | 87.83 $^{b}$ | 89.51 $^{ab}$ | 88.80 $^{ab}$ | 2.66 | 0.002 | ns | ns | ns | ns | ns |
| Backfat depth, cm | 386 | 5.26 $^{a}$ | 5.29 $^{a}$ | 5.17 $^{ab}$ | 5.16 $^{ab}$ | 4.62 $^{b}$ | 4.64 $^{b}$ | 4.90 $^{ab}$ | 4.80 $^{ab}$ | 0.70 | ns | ns | 0.0005 | ns | ns | ns |
| Muscular dry matter, % | 386 | 30.32 $^{a b}$ | 31.49 $^{a}$ | 29.89 $^{b}$ | 30.56 $^{ab}$ | 29.66 $^{b}$ | 30.82 $^{ab}$ | 30.32 $^{ab}$ | 30.37 $^{ab}$ | 5.49 | ns | 0.02 | ns | ns | ns | ns |
| Liver dry matter, % | 337 | 30.44 $^{b}$ | 29.78 $^{b}$ | 29.81 $^{b}$ | 29.71 $^{b}$ | 32.45 $^{a}$ | 32.82 $^{a}$ | 30.52 $^{b}$ | 31.53 $^{ab}$ | 4.29 | 0.04 | ns | <.0001 | ns | ns | ns |
| Muscular drip loss, % | 325 | 6.92 $^{a}$ | 3.83 $^{b}$ | 5.53 $^{ab}$ | 5.62 $^{ab}$ | 5.65 $^{a b}$ | 4.59 $^{ab}$ | 6.42 $^{a}$ | 6.34 $^{a}$ | 2.64 | ns | t | ns | t | ns | ns |
| Intramuscular fat, % | 387 | 27.75 $^{a b}$ | 28.04 $^{ab}$ | 24.37 $^{ab}$ | 26.91 $^{ab}$ | 24.01 $^{a b}$ | 29.22 $^{a}$ | 22.75 $^{b}$ | 25.78 $^{ab}$ | 6.45 | t | 0.03 | ns | ns | ns | ns |
| Liver fat, % | 345 | 18.00 $^{c}$ | 24.46 $^{a}$ | 20.48 $^{bc}$ | 20.80 $^{bc}$ | 19.60 $^{b c}$ | 21.93 $^{ab}$ | 21.08 $^{b}$ | 20.36 $^{bc}$ | 2.74 | ns | 0.004 | ns | 0.002 | ns | t |

BW= Birth weight, Nutr= Maternal Nutrition. LBW= Low birth-weight, NBW= Normal birth-weight. RMSE = root-mean-square error. Ns= not significant, t= 0.1>*P*>0.05. Different letters in a line indicate significant differences (*P*<0.05).

### Evaluation of plasma indexes of glucose and lipids metabolism

The plasma indexes of glucose and lipid metabolism at adulthood were mainly affected by the maternal nutritional status during pregnancy. At 215 days old, the pigs of the underfed group showed lower plasma concentrations of glucose and HDL-c than pigs of the control group (Fig. 4; $P<0.005$ and $<0.0001$, respectively), but higher values for fructosamine and LDL-c ($P<0.005$ and $<0.05$, respectively). At slaughter, a sex-related effect, independently from maternal diet and BW, was observed on triglyceride concentrations, which were higher in females than in males (42.4±12.9 mg/dL vs. 38.9±12.5 mg/dL, respectively; $P<0.05$).

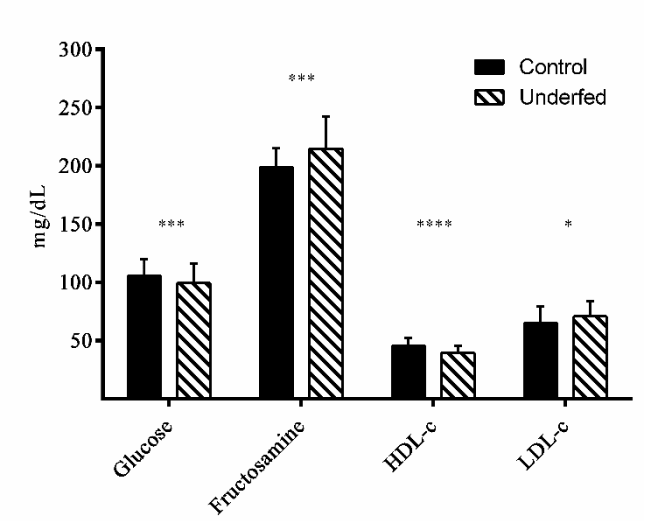


**Fig. 4: Effect of maternal feed restriction on metabolic status at 215 days old.** Mean ± SD values of glucose, fuctosamine, HDL-c and LDL-c concentrations in control and underfed pigs. Asterisks indicate significant differences between groups (*=P<0.05, **=P<0.01, ***=P<0.005, ****=P<0.0001).

### Assessment of carcass features and evaluation of drip-loss and moisture

Carcass features, meat quality and characteristics of liver tissue were significantly affected by maternal nutritional status, BW, and sex (Table 1). In brief, control pigs had higher carcass weight and yield (122.0±6.1 vs. 118.1±9.7 kg and 79.3±2.2 vs. 78.1±1.7 %; $P<0.0001$ for both) and higher backfat depth than underfed pigs (5.2±0.7 vs. 4.8±0.7 cm, respectively; $P<0.0005$). Birth-weight affected carcass weight and length, which were greater in NBW than in LBW pigs (120.3±7.9 vs. 116.5±12.4 kg and 89.7±2.8 vs. 88.0±2.2 cm, respectively; $P<0.005$ for both), while sex affected muscular dry matter and LD fat content with a higher amount in males than in females (30.5±1.9 vs. 30.1±1.4 % and 26.6±6.8 vs.

23.6±6.1 %, respectively; *P*<0.05 for both). Low BW-males also showed the largest liver fat content (22.7±3.8 %; *P*<0.005 for the interaction effect). Moreover, the dry matter of liver samples was lower in NBW (30.5±2.0 vs. 31.7±2.2 %, respectively; *P*<0.05) and control pigs (29.8±0.9 vs. 31.1±2.4 %, respectively; *P*<0.0001) than in LBW and underfed groups, respectively.

**Evaluation of the fatty acid composition of the tissues**

Maternal nutritional status and offspring sex were the main factors affecting fatty acid (FA) profile of the different tissues (liver, LD and subcutaneous fat).

In the liver (Supplementary Table 4, Additional File 5), maternal nutritional status had more effects on FA composition of the Polar Lipid (PL) fraction than on the Neutral Lipid (NL) fraction. The assessment of the PL fraction showed that underfed pigs had greater concentrations of C16:1n-9, C18:1n−9 and MUFA (0.3±0.1 vs. 0.25±0.1, 17.7±1.9 vs. 16.4±2.3 and 20.8±2.3 vs. 19.3±2.7 g/100g, respectively) and higher desaturase indexes (C18:1/C18:0 and MUFA/SFA; 0.66±0.1 vs. 0.6±0.1 and 0.43±0.1 vs. 0.39±0.1, respectively), but lower concentrations of C22:4n−6 than control pigs (0.7±0.2 vs. 0.8±0.2 g, respectively; *P*<0.05 for all the values). The composition of PL fraction was also affected by BW, with higher C18:0 concentration in LBW than in NBW pigs (30.6±1.8 vs. 29.5±2.1 g/100g; *P*<0.05). There were also sex-related effects since females showed higher C18:2n−6 concentrations and lower C18:3n−3 values than males (12±1.1 vs. 11.7±1.0 and 0.23±0.1 vs. 0.25±0.1 g/100g, respectively*;* *P*<0.05 for both). Moreover, control-LBW-females showed the lowest values of C16:0 and LBW-males had the highest C17:1 levels in this fraction (0.27±0.1 g of C17:1/100g; *P*<0.05 for the interaction effects). On the other hand, the assessment of the NL fraction showed higher C20:1n−9 and C20:3n−6 concentrations and ∑n−6/∑n−3 ratio in the control group than in the underfed group (0.7±0.3 vs. 0.4±0.1, 0.5±0.2 vs. 0.4±0.2 g/100g and 9.4±1.2 vs. 8.3±1.0, respectively; *P*<0.01 for all). Furthermore, control pigs had lower C18:3n−3 and C22:6n−3 values than underfed individuals (0.3±0.1 vs. 0.4±0.1 and 0.9±0.3 vs. 1.1±0.3 g/100g, respectively; *P*<0.05). In the NL fraction, the values of C16:1n−9 and C20:3n−6 showed a triple interaction (*P*<0.05 for both). No sex effects were found.

In LD muscle (Supplementary Table 5, Additional File 6), main differences were also observed in the PL fraction due to the influence of maternal diet and offspring BW. The assessment of the PL fraction showed higher concentrations of C18:1n−9, n-3 FA, MUFA, and C20:1 n−9 (15.4±2.3 vs. 13.5±1.5, 3.6±0.5 vs. 3.1±0.2, 21.6±2.8 vs. 21±1.8 and 0.33±0.1 vs. 0.26±0.1 g/100g, respectively), lower levels of C16:1n−9 and SFA (0.3±0.1 vs. 1.3±0.3 and

31±1.6 vs. 32.9±1.7 g/100g, respectively) and lower ∑n−6/∑n−3 ratio in control than in underfed pigs (12.4±1.3 vs. 13.7±1.0, respectively; *P*<0.005 for all). Maternal nutritional status also significantly affected the desaturase indexes in the PL fraction, with these indexes being higher in control pigs than in underfed pigs (C18:1/C18:0: 2.1±0.3 vs. 2±0.2 and MUFA/SFA: 0.7±0.1 vs. 0.63±0.1; *P*<0.05 for both). Birth-weight affected the concentration of PUFA in the control group (47.5±3.5 vs. 46±2.7 g/100g; *P*<0.05) and, overall, PUFA levels were higher in control-NBW pigs than in their control-LBW littermates (47.7±3.3 and 45.2±4.0 g/100g; *P*<0.01 for the interaction effects). Finally, underfed-LBW pigs had lower C18:0 and C18:1n−9 values than control-LBW (8.3±0.7 and 9.3±0.6 and 13.7±1.5 and 16.8±3.0 g/100g, respectively) and control-NBW pigs showed the highest C18:2n-6 concentration (29.7±2.2 g/100g; *P*<0.05 for the interaction effects). There was also an interaction of maternal diet and BW in values of C16:1n-9, C22:5n−3, MUFA, n-3 FA, n-6 FA and unsaturation index. On the other hand, the assessment of the NL fraction showed higher levels of C16:1n−9, C18:1n−7 and C20:4n−6 and higher ∑n−6/∑n−3 ratio in the NL fraction (0.21±0.0 vs. 0.17±0.0, 4.8±0.6 vs. 3.5±0.6, 0.14±0.1 vs. 0.13±0.1 g/100g and 4.4±0.8 vs. 4.1±0.7, respectively; *P*<0.05 for all) in control pigs than underfed pigs. Conversely, control pigs showed lower C18:3n−3 and C18:1n−9 values than underfed pigs (0.46±0.0 vs. 0.5±0.0 and 47.9±2.2 vs. 49.4±2.0 g/100g; *P*<0.0001 for both).

In subcutaneous fat (Supplementary Table 6, Additional File 7), conversely to liver and muscle, the main effects were driven by offspring sex. Males had higher desaturase activity (C18:1/C18:0: inner: 4±0.7 vs. 3.7±0.5 g/100g and outer: 4.6±0.7 vs. 4.3±0.5; MUFA/SFA: inner: 1.3±0.2 vs. 1.2±0.1 g/100g and outer: 1.5±0.1 vs. 1.4±0.1) and MUFA concentration than females, both in inner and outer layers (inner: 52.4±2.4 vs. 51.2±1.7 g/100g and outer: 53.9±2 vs. 52.8±1.7 g/100g, respectively; *P*<0.005 for all), and a higher unsaturation index in the inner layer (0.7±0.0 vs. 0.69±0.0, respectively; *P*<0.01) than females. Conversely, females had greater SFA values at the inner layer (40.6±1.8 vs. 39.4±2.6 g/100g, respectively; *P*<0.01) and higher PUFA concentration at the outer layer (9.6±0.9 vs. 9.4±0.7 g/100g, respectively; *P*<0.005) than males. Maternal diet also affected FA composition of both inner and outer layers. In the outer layer, underfed pigs had lower SFA values (36.6±1.8 vs. 37.8±2.2 g/100g, respectively; *P*<0.0001) and higher MUFA and PUFA concentrations and unsaturation (53.9±1.6 vs. 52.9±2.0, 9.6±0.9 vs. 9.4±0.7 g/100g and 0.74±0.0 vs. 0.72±0.0, respectively) and desaturase indexes than control pigs (C18:1/C18:0: 4.6±0.6 vs. 4.3±0.6 and MUFA/SFA: 1.5±0.1 vs. 1.4±0.1; *P*<0.005 for all). In the inner layer, underfed pigs had higher ∑n−6/∑n−3 ratio than control pigs (11.8±4.2 vs. 8.6±0.3, respectively; *P*<0.0001). Finally, the assessment of possible interactions showed

that control-LBW pigs had lower n-6, n-3 and PUFA concentrations (8.5±1.0, 0.63±0.1 and 9.1±0.9, respectively) than underfed-LBW counterparts at the outer layer (9.2±1.1, 0.67±0.1 and 9.9±1.2, respectively; $P<0.05$, for all the interaction effects). There were triple interactions in the inner layer related to values of C18:1n9, SFA, MFA and unsaturation index and in the outer layer related to C14:0 and C17:0.

## Discussion

The results of the present study indicate that a light maternal feed restriction during mid pregnancy has no effects on sow productivity and piglet phenotype (body weight and size) at birth and weaning. Hence, at first glance, such nutritional management would be adequate for diminishing costs of production. However, the assessment of late postnatal development evidenced that offspring from feed-restricted sows have a higher mortality rate, metabolic disturbances and worse growth patterns with longer time-periods for achieving target-weight and poorer carcass and meat quality traits. Offspring sex and BW modulated these effects, consequently, maternal feed restriction would finally penalize the profitability of the farm.

### Effects of maternal feed restriction on characteristics of litters and piglets at birth and weaning

Maternal feed restriction did not affect the total number of born piglets born nor the mean number of live, stillborn and mummified piglets. Concurrently, there were no differences in birth-weight of piglets from control and underfed groups at farrowing but, on the contrary, there was a lower incidence of LBW piglets in underfed than in control sows.

The higher incidence of LBW piglets in control group may be related to the existence of a higher number of litters with 10-13 piglets in the control group and therefore a greater incidence of LBW in the more prolific litters [3, 4]. The lack of differences in sow productivity and piglet weight may be related to the degree (intake of 70% of the daily requirements) and timing of feed restriction (days 38 to 90 of pregnancy). There are previous studies in lean breeds addressing that a modest reduction in the dietary intake did not affect reproductive parameters of the sows or piglets [2, 33]. Concomitantly, the timing of feed restriction was also determinant for the lack of effects on sow productivity found in the current study. Restriction was started on day 38 of gestation (i.e., after

completion of implantation and placentation and beginning of early fetal stage at day 35 of pregnancy, when the most of conceptus losses occurs; [34]) and stopped at day 90 of pregnancy (i.e., early in the third period of gestation, just before fetuses enter the final growth phase when fetal nutrient demand is greatest; [35]). These effects, described in lean breeds, would be even less critical in the Iberian and other traditional breeds due to their high availability of energy stored in fat depots and their ability to mobilize fat depots in the case of undernutrition [9].The absence of feed restriction during the last days of pregnancy and lactation may also be contributing to the lack of differences in the number of weaned piglets and the total litter weight per sow, since feed intake at the end of pregnancy and the suckling phase are determinant for milk production and composition [36].

However, we have to point out that, despite differences in BW, the nutritional status of the sows during pregnancy affected the morphology of their piglets; piglets from restricted sows had longer trunks at birth and narrower abdomens at weaning. These differences warrant further research since body measures may be interesting to identify piglets with reduced growth potential. In fact, our results may support data addressing that abdominal perimeter may correlate better with postnatal performance than BW, especially during the suckling phase [37].

**Effects of maternal feed restriction and offspring birth-weight and sex on postnatal development**

The interaction between maternal nutritional status and offspring birth-weight, modulated by sex, has a prominent effect on postnatal development. At weaning, control-NBW piglets had higher values for body weight, ADWG and backfat depth than the other groups. We have to point out that underfed-LBW piglets evidenced catch-up growth during suckling period, with higher ADGW values than the control-LBW group. However, such catch-up growth was mainly due to a higher fat deposition, which supports previous studies addressing the existence of IUGR effects on appetite-regulation and metabolic pathways [10, 38].

In the growing phase, control-LBW piglets of both sexes caught up with control-NBW pigs. Such pattern of catch-up growth may be related, in this case, to the enrichment of pathways involved in protein deposition and cellular growth [39]. Control-LBW-females had the highest ADWG at 110 days old, despite lowest ADWG at weaning. This catch-up growth could be a mechanism to balance an early low growth. Conversely, underfed-

LBW-females showed the lowest ADWG, which reinforces the deleterious effects of maternal feed restriction on offspring development, especially in females.

At later stages, from 150- to 180 days old, males had better growth parameters than females in agreement with data obtained in both modern and traditional breeds [40, 41] and LBW males grew less efficiently than NBW males. In fact, all female groups had lower ADWG than their respective male counterparts; such effect also interacted with BW at slaughter suggesting that both control- and underfed-LBW-female piglets never recovered from a slower growth pattern. Maternal feed restriction also affected growth patterns during the fattening phase. At 215 days old, overall, control pigs showed faster growth and lower FCR than underfed pigs.

Finally, the overall ADWG and the slaughter age was again determined by significant interactions among maternal diet and offspring BW and sex. In brief, pigs from control sows and NBW had better growth-efficiency than their counterparts, in agreement with previous data in lean breeds [11, 42, 43]. Such effects were modulated by sex since males reached heavier final weights, with control and underfed LBW-females showing the lightest values. In conclusion, our results support previous studies addressing more efficient growth patterns in NBW pigs [44, 45].

The differences in growth patterns between groups were concomitant to differences in body composition, in the patterns of muscle development and fat deposition, which may finally affect carcass yield and meat quality. At weaning, underfed and LBW piglets had smaller loin diameters than their counterparts, which may be related to effects from intrauterine restriction, either of maternal or placental origin respectively, on the development of secondary myofibers. The development of these fibers occurs from days 55 to 90 of gestation and it has been described to be modulated by epigenetic nutritional factors [46]; hypothesis reinforced by our results. However, the differences between control and underfed and NBW and LBW piglets were lost afterward, which may indicate a fiber hypertrophy during postnatal growth similar to that described in lean breeds [47, 48]; hypertrophy being stronger in males in all the groups. However, these hypotheses cannot be elucidated with the design of the current trial and further studies are warranted.

Pigs from restricted pregnancies showed a higher trend for adiposity, evidenced by higher values for backfat depth throughout postnatal development, which penalized carcass quality and feed conversion efficiency. Such trend for adiposity is related to the prenatal programming evidenced in these individuals [49] due to a catch-up growth mainly based

on fat deposition [50]. Moreover, backfat deposition in underfed pigs were mostly related to a thicker inner layer. Therefore, underfed and control pigs might have different metabolic patterns; a hypothesis confirmed by data from metabolic status at adulthood.

**Effects of maternal undernutrition and offspring birth-weight and sex on metabolic status and carcass and FA composition**

The results of the present study supported the existence of differences in both glucose and lipid metabolism between pigs from control and restricted pregnancies. The pigs from restricted pregnancies showed higher concentrations of fructosamine and lower levels of HDL-c but higher of LDL-c (dyslipidemia). These parameters evidence a higher trend for insulin resistance in these individuals, following suboptimal nutrition in utero, catch-up growth and higher fat deposition [38, 51]. Adiposity increases insulin resistance [52] and, in these individuals, higher fructosamine levels evidenced high glucose levels over time while dyslipidemia has also been linked to obesity with insulin resistance [53]. We finally have to highlight a sex-related effect in triglyceride concentrations, which were higher in females. The same effect was previously found in purebred Iberian pigs after prenatal programming [17]. Hypertriglyceridemia may also be indicative of impaired glucose tolerance [54, 55].

The data from carcass traits, concomitantly with other data described above, showed better development in control and NBW pigs during the growing-finishing phase. Control pig had higher carcass yields, despite a lack of significant differences in body weight between control and underfed pigs at slaughter. This may indicate a greater development of internal structures in underfed pigs, such as organs or visceral fat, in agreement with prenatal programming and previous data in purebred Iberian pigs [10, 17].

Data evidencing metabolic disturbances were reinforced by the FA composition analysis of liver. This analysis showed significant effects of nutritional status and mainly, in the PL fraction (i.e., there were more differences in the membrane composition than in storage lipids; [56], which may be related to metabolic disturbances and lipotoxicity. In the PL fraction, concentrations of MUFA and C18:1n–9, which is its main FA, were greater in underfed pigs and, consequently, desaturation indexes were too. These indexes are linked to SCD1 activity indicating an increase of lipogenesis and possibly metabolic disorders [31, 57, 58]. Moreover, a high content of C16:1 (product of SCD1), as we found in underfed pigs, is considered a significant marker of adiposity and insulin dysregulation [59]. Sex-related effects in liver PL fraction were linked to C18 unsaturated FA, highlighting greater

C18:2n–6 value and lower C18:3n–3 concentrations in females. Both FA are essential FA and necessary for metabolic processes.

In LD muscle, PL fraction showed more changes due to maternal feed restriction than the storage fraction (NL). Herzberg et al. [60] studied the changes in FA composition that occur during fasting, which mainly focus on the selective mobilization. These authors showed the selective mobilization importance in the storage fraction, whereas in our experiment the main changes occur in the PL fraction with a minor content of C18:0 and n-3 FA in pigs from underfed sows during pregnancy. Moreover, the selective mobilization patterns are different to preserve certain PUFA in special situations as hibernating mammals [61]. However, in our study, underfed pigs showed a lower content of C18:0 and higher content of C18:3 n-3 and C18:1 n-9 than control pigs in the NL fraction of LD. This FA profile may be related to the content of oxidative muscle fibres [62]. Moreover, these differences could indicate a different mobilization pattern in a light maternal feed restriction during pregnancy than in fasting or hibernating situations.

The analysis of backfat depth, both in the outer and inner layer showed that underfed pigs had a higher desaturation index than controls, which would support previous evidence of metabolic differences in individuals with maternal feed restriction [63]. Moreover, underfed pigs showed greater C18:2 n-6 concentrations in the outer layer and lower levels of C18:0 levels in both layers. The levels of C18:2 n-6 are significant for the production of quality meat products because high values are associated with rancidity problems and impaired water migration [64].

## Conclusion

In conclusion, the present experiment showed the absence of effects of feed restriction during mid gestation on the total number of piglets born or in the mean BW of the piglets at farrowing. However, the adverse effects of maternal nutritional restriction became evident with increasing offspring age and were related to impaired growth patterns and altered carcass quality and FA composition. Control pigs had better growth patterns and feed conversion efficiency than underfed pigs.

Therefore, maternal nutrition during pregnancy has a critical effect on the productive parameters of their offspring, which is even more important in high-quality production

systems with a long finishing phase as used in the Iberian pig and similar traditional breeds.

## Abbreviations

ADWG: Average daily weight gain
BW: Birth-weight
FA: Fatty acid
FCR: Feed conversion ratio
HDL-c: High-density lipoprotein cholesterol
IUGR: Intrauterine growth restriction
LBW: Low birth-weight
LD: Longissimus dorsi
LDL-c: Low-density lipoprotein cholesterol
MUFA: Monounsaturated fatty acids
LS: Litter size
NBW: Normal birth-weight
NL: Neutral lipids
PL: Polar lipids
PUFA: Polyunsaturated fatty acids
SCD1: Stearoyl-CoA desaturase enzyme 1
SD: Standard deviation
SFA: Saturated fatty acids
RMSE: Root mean square error

## Declarations

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**Authors' contributions**

BI, CO, MVG and AGB designed research. MVG, CGC, SA, LTR, CO, AGB and BI collected data and samples. MVG and LTR conducted the laboratory analyses. MVG and BI analyzed data. MVG, BI and AGB wrote the original draft. SA, LTR, CGC and CO reviewed and edited the paper. All authors read and approved the final manuscript.

**Ethics approval and consent to participate**

The experimental procedures were assessed and approved (report CEEA 2012/036) by the INIA Committee of Ethics in Animal Research (the Institutional Animal Care and Use Committee).

**Availability of data and materials**

The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

**Competing interests**

The authors declare that they have no competing interests

**Consent for publication**

Not applicable.

## List of Additional Files

Additional File 1. PDF. **Reliability criteria for biochemical plasma assays.** Tabular data.

Additional File 2. XLS. **Supplementary Table 1. Calculated analysis (g/kg, dry-matter basis) and fatty acid composition of the diets**. Tabular data.

Additional File 3. XLS. **Supplementary Table 2: Phenotypic parameters at birth and weaning.** Tabular data.

Additional File 4. XLS. **Supplementary Table 3: Growth during growing-fattening phase.** Tabular data.

Additional File 5. XLS. **Supplementary Table 4: Fatty acids composition of liver (g/100 g total fatty acids).** Tabular data.

Additional File 6. XLS. **Supplementary Table 5: Fatty acids composition of longissimus dorsi muscle (g/100 g total fatty acids).** Tabular data.

Additional File 7. XLS. **Supplementary Table 6: Fatty acids composition of subcutaneous fat (g/100 g total fatty acids).** Tabular data.

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## 4.3. Experimento 3

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### Polyphenols and IUGR pregnancies: Maternal hydroxytyrosol supplementation improves prenatal and early-postnatal growth and metabolism of the offspring

Short title: Hydroxytyrosol supplementation in IUGR pregnancies

Marta Vázquez-Gómez, Consolación García-Contreras, Laura Torres-Rovira, José Luis Pesantez; Ernesto Gómez-Fidalgo, Raúl Sánchez-Sánchez, Cristina Óvilo, Beatriz Isabel, Susana Astiz and Antonio González-Bulnes.

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**Polyphenols and IUGR pregnancies: maternal hydroxytyrosol supplementation improves prenatal and early-postnatal growth and metabolism of the offspring**

**Short Title:** Hydroxytyrosol supplementation in IUGR pregnancies

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## Abstract

Hydroxytyrosol is a polyphenol with antioxidant, metabolism-regulatory, anti-inflammatory and immuno-modulatory properties. The present study aimed to determine whether supplementing the maternal diet with hydroxytyrosol during pregnancy can improve pre- and early post-natal developmental patterns and metabolic traits of the offspring. Experiment was performed in Iberian sows fed a restricted diet in order to increase the risk of IUGR. Ten sows were treated daily with 1.5 mg of hydroxytyrosol per kg of feed between Day 35 of pregnancy (30% of total gestational period) until delivery whilst 10 animals were left untreated as controls. Number and weight of offspring were assessed at birth, on post-natal Day 15 and at weaning (25 days-old). At weaning, body composition and plasma indexes of glucose and lipids were measured. Treatment with hydroxytyrosol was associated with higher mean birth weight, lower incidence of piglets with low birth weight. Afterwards, during the lactation period, piglets in the treated group showed a higher body-weight than control piglets; such effects were even stronger in the most prolific litters. These results suggest that maternal supplementation with hydroxytyrosol may improve pre- and early post-natal development of offspring in pregnancies at risk of IUGR.

**Keywords**: antioxidants; hydroxytyrosol; IUGR, polyphenols; pregnancy; fetal programming.

## Introduction

Inadequate maternal nutrition and/or placental efficiency can result in insufficient supply of oxygen and nutrients to the fetus, causing intrauterine growth restriction (IUGR) and leading to the birth of small-for-gestational-age (SGA; also known as low-birth-weight, LBW) offspring [1, 2].

In humans, LBW is associated with increased risk of perinatal morbidity and mortality, accounting for 800,000 neonatal deaths worldwide [3]. Moreover, surviving offspring are predisposed to lifelong chronic non-communicable disorders such as obesity, type II diabetes and cardiovascular diseases [4-6].

In veterinary medicine and animal production, perinatal deaths due to LBW offspring are also significant, but even more concerning are the substantial economic losses to farms. In addition to lost productivity, IUGR can reduce the value of farm products by introducing undesirable heterogeneity: low-birth-weight animals show lower growth potential, lower feed efficiency, lower meat yield and excess adiposity relative to their normal-weight littermates, giving rise to heterogeneous growth patterns, carcass conformation and meat characteristics among individuals in the same litter and feedlot [7-10]. These differences penalize the value of the product since the market requires products that are uniform in weight, conformation and composition.

In consequence, there is a strong necessity of strategies and tools for alleviating incidence and consequence of IUGR, both in human and veterinary medicine. IUGR involves not only inadequate nutrient supply but also reduced oxygenation, which causes hypoxia that increases oxidative stress and triggers low-grade inflammation [11]. The antioxidant defense system in IUGR fetuses is weakened, exacerbating oxidative stress [12-14], which in turn aggravates the effects of IUGR [15]. Previous studies in sheep have shown that the deleterious effects of oxidative status at the fetoplacental unit may be prevented by the administration of antioxidant agents (e.g.: antioxidant vitamins and melatonin), which ameliorates the antioxidant/oxidative status, improves the placental function and increases the weight and viability of the newborn [16-19].

The present trial aimed to study the effects, on pregnancies affected by IUGR, of a different source of antioxidant agents than vitamins, i.e. polyphenols. Polyphenols are common constituents of many plant-derived foods (i.e.: pieces or derivatives of fruits, vegetables and seeds), and the most abundant dietary antioxidants. Specifically, we are interested in studying the possible usefulness of the maternal supplementation with hydroxytyrosol. Hydroxytyrosol is a polyphenol present in olive fruits (and, hence, in virgin olive oil) with not only a prominent antioxidant activity, but also metabolism-regulatory, anti-inflammatory and immuno-modulatory properties [20]. These benefits are boosted by high stability, degree of absorption and bioavailability of the active substance [21]. In consequence, there is increasing clinical and epidemiological evidencesevidence on its relevance against pathologies such as cancer, cardiovascular, metabolic and neurodegenerative diseases [22].

However, possible usefulness of polyphenols for reproductive health and pregnancy is only beginning to be explored [23] Hence, we aimed to determine whether hydroxytyrosol

supplementation of maternal food during pregnancy may improve pre- and early post-natal developmental patterns and metabolic traits of the offspring by using a translational swine model previously developed in our laboratory [8, 24].

## Material and methods

### Ethics statement

The study was performed according to the Spanish Policy for Animal Protection RD53/2013, which meets the European Union Directive 2010/63/UE about the protection of animals used in research. The study protocol was specifically assessed and approved by the INIA Committee of Ethics in Animal Research (report CEEA 2012/036), which is the named Institutional Animal Care and Use Committee (IACUC) for the INIA. Sows were housed in INIA animal facilities, which meet local, national and European requirements for Scientific Procedure Establishments.

### Experimental design

The study involved 20 primiparous Iberian sows that became pregnant after cycle synchronization with altrenogest (Regumate®, MSD, Boxmeer, The Netherlands) and insemination with cooled semen from a purebred Iberian boar.

Sows were fed a standard grain-based diet with mean content values of 89.8% of dry matter, 15.1% of crude protein, 2.8% of fat, and 12,56MJ of Metabolizable Energy/Kg. From the start of the experimental period until gestational Day 35, food amount was adjusted to fulfill individual daily maintenance requirements based on data from the British Society of Animal Science [25]. On gestational Day 35, all sows were weighed and the food amount from that day until delivery was adjusted to fulfill 50% of daily maintenance requirements. This diet restriction has been previously found to affect fetal development and to induce lower birth-weight in the newborns [8, 24]. Also on gestational Day 35, sows were pair-matched according to body-weight and 10 females remained as untreated control group (group C) whilst the remaining 10 females (group HT) acted as the treated group by receiving 1.5mg of hydroxytyrosol per kg of feed each day from Day 35 of pregnancy to delivery.

### Assessment of morphological features and early post-natal development of piglets

At birth, the total number of live and stillborn piglets in each litter was recorded, together with the sex and weight of all piglets. Piglets with LBW were defined as those with a birth-weight lesser than one standard deviation of the mean value of the control littermates after adjusting for sex [26]. In addition, the percentage of piglets with body weights less than 1 kg was recorded; this weight threshold is commonly used to identify animals at higher risk of perinatal mortality [27]. All live piglets were tagged with earrings and underwent within-group fostering in order to equalize the number of piglets among sows. Piglets remained with sows in pens (one sow per pen) until weaning at 25 days-old. The number and causes of death were recorded from birth to weaning. Living piglets were weighed at 15 and 25 days-old. Average daily weight gain (ADWG) was determined for the total period of lactation as well as for two intermediate periods (from post-natal Day 0 to Day 15 and from Day 15 to Day 25). ADWG was calculated using the formula: [final weight - initial weight] / number of days.

### Assessment of body composition of piglets

At weaning, piglets were euthanized using CO2, stunned and exsanguinated in compliance with standard procedures stipulated in Spanish regulation RD53/2013. Back-fat depth and loin diameter were measured immediately at the P2 point, at the level of the head of the last rib, using an ultrasound machine with a multifrequency linear array probe (SonoSite S-Series, 5-8 MHz; SonoSite Inc., Bothell, WA, USA). Then, the head was separated from the trunk at the atlanto-occipital union and weighed in order to determine the ratio of head-to-body weight. Afterwards, all viscerae were removed and weighed together. Finally, major organs (brain, heart, lungs, liver, intestine, kidneys, spleen, pancreas and adrenal glands) were weighed individually for assessing possible patterns of asymmetrical IUGR. The following weight-ratios were considered: weight of brain, heart, lungs, liver, kidneys, intestine, pancreas, spleen and adrenals relative to total viscera weight.

### Assessment of the metabolic status of piglets

Blood sampling was performed, during sedation, by puncture of the vena cava cranealis using sterile 5-ml EDTA vacuum tubes (Vacutainer Systems Europe; Becton Dickinson, Meylan Cedex, France). Blood samples were centrifuged at 1500g for 15min and the

plasma was stored in polypropylene vials at -20°C until assayed for determination of parameters related to metabolism of glucose and lipids.

Parameters for glucose (glucose and fructosamine) and lipids profiles (triglycerides, total cholesterol, high-density lipoproteins cholesterol [HDL-c] and low-density lipoproteins cholesterol [LDL-c]) were assessed with a clinical chemistry analyzer (Saturno 300 plus, Crony Instruments s.r.l., Rome, Italy), according to the manufacturer's instructions.

### Statistical analyses

T-student tests were used to assess the effects of independent variables (maternal diet and piglet sex) on litter size, occurrence of IUGR and LWB and percentage of piglets below 1Kg BW). For statistical purposes, litter size was afterwards categorized in two groups (2-6 vs >6 piglets/litter), based on the mean litter size for the Iberian breed (6.5 piglets [28], or into three groups (2-6, 7-8 vs 9-10 piglets/litter) for assessing possible effects in the most prolific litters. Dependent variables related to offspring phenotype (weight, ADWG, back-fat depth, muscle diameter, organ development and metabolic indexes) were assessed using two-way ANOVA in a General Linear Model; interactions among potential confounding factors were observed and fixed when statistically significant ($P<0.05$; life time, litter size, maternal diet and offspring sex and their interaction). Differences between male and female piglets and between litter sizes in body weight, organ development and metabolic indexes were assessed using t-student test. Finally, changes over time were assessed by ANOVA for repeated measures with the Greenhouse-Geisser correction. All the results were expressed as mean ± SEM, and statistical significance was accepted from $P < 0.05$, while P-values between 0.05 and 0.09 were considered to indicate a tendency.

## Results

### Effects of hydroxytyrosol supplementation on birth-weight and -size and early postnatal development of the piglets

The hydroxytyrosol supplementation did not significantly affect litter size (group HT: 6.7±0.1 piglets, range of 2-10; group C: 5.8±0.1 piglets, range of 2-10), but it was related to significant increases in BW of the piglets at birth and during the postnatal period.

The group HT was characterized by a higher mean birth-weight than the group C (1.3±0.1 vs 1.2±0.1 Kg, respectively;, P < 0.001), lower incidence of LBW (6.3 vs 13.2%, respectively; P < 0.05) and lower incidence of individuals with a birth-weight lower than 1Kg (6.3 vs 17.0%, respectively; P < 0.05). In both groups, these percentages were numerically lower in female than in male piglets (group HT: 4.0 vs 7.9% and group C: 9.5 vs 18.7%, respectively; P = 0.15).

The effects of hydroxytyrosol treatment on birth-weight were determined by litter size and offspring sex (Tables 1 and 2). Overall, the group HT had higher BW values than the group C as previously described (P<0.001), the neonates were heavier in litters with a lower number than seven piglets (P<0.01) and males were heavier than females (P<0.05). As a result of both of these influences, male piglets in the group HT were significantly heavier than C male counterparts and HT female littermates (P<0.05). Females in the hydroxytyrosol group tended to be heavier than female controls (P=0.06), but this difference did not achieve statistical significance.

**Table 1. Differential effect of hydroxytyrosol supplementation on birth weight of male and female piglets.** Mean values (±SEM) of birth-weight (kg) in female and male piglets from hydroxytyrosol-treated and control groups.

| | Females | Males |
|---|---|---|
| **Control** | $1.10 \pm 0.04^{Aa}$ | $1.19 \pm 0.03^{Bc}$ |
| **Hydroxytyrosol** | $1.20 \pm 0.03^{Cb}$ | $1.30 \pm 0.03^{Dd}$ |

Lowercase superscript letters denote significant differences between treatment and control groups: a≠b, 0.09 > P > 0.05; c≠d, P < 0.05. Uppercase superscript letters denote significant differences between sexes: A≠B, 0.09 > P > 0.05; C≠D, P < 0.05.

**Table 2. Differential effect of hydroxytyrosol supplementation on birth weight of piglets from smaller or larger litters.** Mean values (±SEM) of birth-weight (kg) in litters with 2-6 and 7-10 piglets in hydroxytyrosol-treated and control groups.

| | Control | Hydroxytyrosol |
|---|---|---|
| **2-6 piglets** | $1.21 \pm 0.05^{C}$ | $1.38 \pm 0.05^{D}$ |
| **7-10 piglets** | $1.13 \pm 0.03^{C}$ | $1.23 \pm 0.02^{D}$ |
| **All litters** | 1.15 ± 0.02 | 1.27 ± 0.02 |

Superscript letters denote significant differences between litter sizes: C≠D, P < 0.05.

The positive effects of maternal hydroxytyrosol administration on the birth weight of piglets continued during the lactation period (Fig 1A). Piglets in the hydroxytyrosol group were significantly heavier than control piglets at 15 days (P<0.0001) and 25 days of age (P<0.05). Weight was similar between sexes within each group at each time point, but males in the group HT were significantly heavier than control males at 15 days (P<0.001) and 25 days (P<0.05) The changes in BW were significantly different between groups in piglets from the extreme litters (2-6 and 9-10 piglets; Figs 1B and 1D), but not in piglets from litters with 7-8 individuals (Fig 1C). As a result, body weight at weaning was similar for piglets in the hydroxytyrosol and control groups that were in litters with less than 9 piglets, but maternal hydroxytyrosol supplementation favored the growth of the individuals from litters of 9-10 newborns, specially between 15 and 25 days of age (P<0.01). Hence, at weaning, there was a difference of around 1Kg between these piglets in the groups HT and C and the group HT had more homogeneous body weight at weaning than the group C (Figs 1E and 1F).

The analysis of changes in the average daily weight gain (ADWG) showed concomitant changes through the lactation period. Overall, there were no significant differences in the mean value of ADWG between groups during the first 15 days. However, ADWG increased between 15 and 25 days to a greater extent in control piglets (Fig 2A), with a significant interaction between treatment and litter-size (P<0.01). These findings were related to a significant interaction among treatment, litter size and age (P<0.01). In this way, the evolution of ADWG was higher in the control group in litters with 2-6 piglets (Fig 2B), similar between treatments in litters with 7-8 piglets (Fig 2C) and higher in the group HT in litters with 9-10 piglets (Fig 2D), with greater ADWG in the group HT due to different within-litter ADWG in both groups (Figs 2E and 2F).

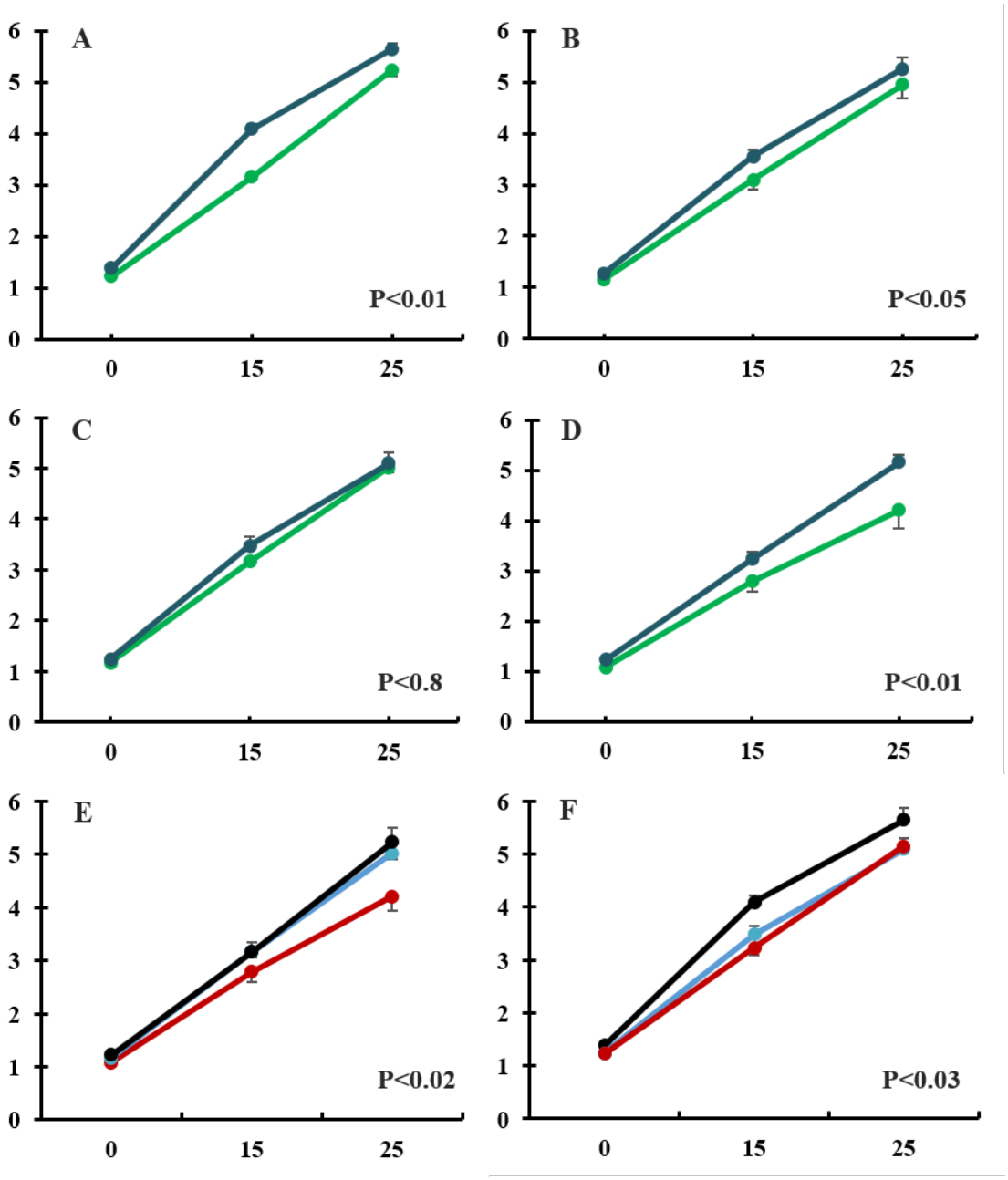


**Fig 1. Changes in body-weight (Kg/day) of piglets throughout the lactation period.** The panel A represents total differences between groups of treatment. Panels B, C and D represent between-treatments differences considering litter size (2-6, 7-8 and 9-10 piglets, respectively). Panels E and F represent within-treatment differences considering litter size in control and treated groups, respectively. Dark blue: Hydroxytyrosol group. Green: Control group. Red: 9-10 piglets/litter. Light blue: 8-7 piglets/litter. Black: 2-6 piglets/litter.

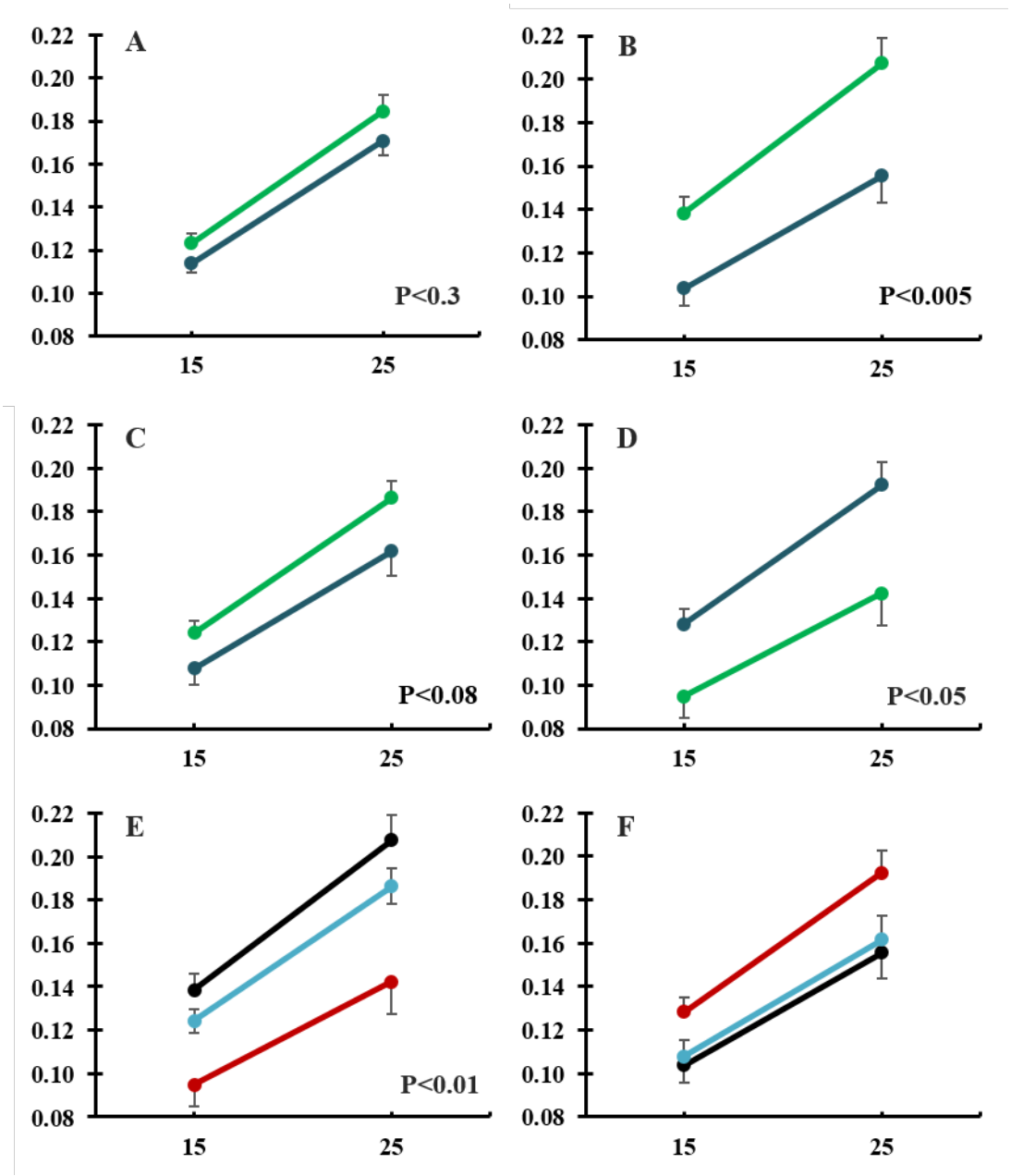


**Fig 2. Changes in ADWG (Kg/day ± S.E.M.) of piglets throughout lactation period.** The panel A represents total differences between groups of treatment. Panels B, C and D represent between-treatments differences considering litter size (2-6, 7-8 and 9-10 piglets, respectively). Panels E and F represent within-treatment differences considering litter size in control and treated groups, respectively. Dark blue: Hydroxytyrosol group. Green: Control group. Red: 9-10 piglets/litter. Light blue: 8-7 piglets/litter. Black: 2-6 piglets/litter.

### Effects of hydroxytyrosol supplementation on body composition of the piglets

At weaning, hydroxytyrosol supplementation was associated with not only greater body weight but also with higher back-fat depth (4.8±0.1 *vs* 3.3±0.1 mm for piglets in the groups HT and C, respectively; P<0.01) as well as higher loin diameter (10.5±0.1 *vs* 8.9±0.1 mm for piglets in the groups HT and C, respectively; P<0.05), independently of sex and litter size.

The assessment of the relative weights of the different organs (Table 3) showed that piglets in the group C had higher relative weights of head-to-total body (P<0.0001) and brain- and liver-to-total viscerae (P<0.0001). There was again a significant interaction between treatment and litter size for the relative weights to total viscerae for kidneys, intestine and pancreas. There were only a significant difference of the relative weight of liver between groups of treatment when comparing piglets from litters with 2-6 littermates, but more ratios were higher in the group C when considering litters with 7-10 neonates, with piglets from more prolific litters in the group C having also higher relative weights of kidneys and lungs (P<0.05 and P<0.01, respectively).

### Effects of hydroxytyrosol supplementation on metabolic status of the piglets

Mean values for indexes of glucose and lipid metabolism at weaning were again affected by a significant in the treatment and litter size (Table 4), without sex-related effects. Among litters with 2-6 piglets, indices were similar between the hydroxytyrosol and control groups. Among litters with at least 7 piglets, plasma concentration of glucose was significantly higher in the hydroxytyrosol group (P<0.05), while the concentration of fructosamine was significantly lower (P<0.001). Again among litters with at least 7 piglets, the hydroxytyrosol group tended to show lower plasma concentrations of total cholesterol (P=0.05), significantly lower LDL-c (P<0.05), significantly higher triglycerides (P<0.05) and similar HDL-c as the control group.

**Table 3. Ratios of organ-to-total viscera weight at weaning.** Mean values (±SEM) of the relative weight (W) of organs to total viscerae-weight (TV-W) for treatment (Hydroxytyrosol, HT vs. Control group, C) and litter size (2-6 vs. 7-10 piglets/ litter).

| | Total | | 2-6 piglets/litter | | 7-10 piglets/litter | |
|---|---|---|---|---|---|---|
| | C | HT | C | HT | C | HT |
| **Brain-W to TV-W** | 0.06 ± 0.001 | 0.05 ± 0.001 | 0.07 ± 0.001$^{a}$ | 0.06 ± 0.001$^{b}$ | 0.06 ± 0.01$^{c}$ | 0.04 ± 0.003$^{d}$ |
| **Liver-W to TV-W** | 0.17 ± 0.001 | 0.15 ± 0.001 | 0.17 ± 0.01$^{c}$ | 0.15 ± 0.003$^{d}$ | 0.17 ± 0.01$^{c}$ | 0.15 ± 0.003$^{d}$ |
| **Pancreas-W to TV-W** | 0.01 ± 0.001 | 0.01 ± 0.001 | 0.01 ± 0.001 | 0.01 ± 0.001 | 0.01 ± 0.002 | 0.01 ± 0.003 |
| **Kidneys-W to TV-W** | 0.04 ± 0.001 | 0.03 ± 0.002 | 0.04 ± 0.001 | 0.03 ± 0.001 | 0.04 ± 0.002$^{c}$ | 0.03 ± 0.002$^{d}$ |
| **Spleen-W to TV-W** | 0.02 ± 0.001 | 0.01 ± 0.001 | 0.02 ± 0.001 | 0.02 ± 0.002 | 0.02 ± 0.002 | 0.01 ± 0.001 |
| **Intestine-W to TV-W** | 0.49 ± 0.01 | 0.48 ± 0.01 | 0.49 ± 0.02 | 0.47 ± 0.01 | 0.48 ± 0.02 | 0.48 ± 0.01 |
| **Heart-W to TV-W** | 0.04 ± 0.002 | 0.04 ± 0.002 | 0.04 ± 0.003 | 0.04 ± 0.001 | 0.04 ± 0.002 | 0.04 ± 0.002 |
| **Lungs-W to TV-W** | 0.09 ± 0.001 | 0.08 ± 0.001 | 0.10 ± 0.01$^{a}$ | 0.08 ± 0.01$^{b}$ | 0.09 ± 0.002$^{e}$ | 0.08 ± 0.002$^{f}$ |

Superscript letters indicate significant differences between groups: a≠b, 0.9 > P > 0.05; c≠d, P < 0.05; e≠f, P < 0.01.

**Table 4. Indexes of glucose and lipid metabolism in piglets at weaning.** Mean plasma concentrations (±SEM) of parameters related to lipid and glucose profile (mg/dl) for treatment (Hydroxytyrosol, HT, vs. Control group, C) and of litter size (2-6 vs. 7-10 piglets/litter).

| | Total | | 2-6 piglets/litter | | 7-10 piglets/litter | |
|---|---|---|---|---|---|---|
| | C | HT | C | HT | C | HT |
| **Glucose** | 148.7 ± 10.5 | 154.2 ± 3.8 | 169.2 ± 29.5 | 152.7 ± 5.8 | 138.4 ± 5.4$^{c}$ | 154.7 ± 4.7$^{d}$ |
| **Fructosamine** | 312.6 ± 11.4 | 270.7 ± 6.9 | 276.2 ± 11.2 | 266.6 ± 15.2 | 330.8 ± 13.5$^{e}$ | 272.2 ± 7.8$^{f}$ |
| **Cholesterol** | 131.0 ± 7.1 | 113.1 ± 5.0 | 123.0 ± 11.6 | 111.3 ± 9.4 | 134.9 ± 9.1$^{a}$ | 113.8 ± 6.0$^{b}$ |
| **HDL-c** | 47.3 ± 2.0 | 49.6 ± 1.6 | 40.8 ± 2.7 | 44.9 ± 2.1 | 50.5 ± 2.2 | 51.3 ± 1.9 |
| **LDL-c** | 70.1 ± 6.4 | 53.8 ± 3.3 | 68.8 ± 8.8 | 54.8 ± 7.8 | 70.8 ± 8.9$^{c}$ | 53.5 ± 3.7$^{d}$ |
| **Triglycerides** | 58.5 ± 8.7 | 90.1 ± 7.0 | 71.2 ± 22.5 | 113.6 ± 14.9 | 52.2 ± 7.0$^{c}$ | 81.7 ± 7.3$^{d}$ |

Superscript letters indicate significant differences between groups: a≠b, 0.9 > P > 0.05; c≠d, P < 0.05; e≠f, P < 0.01.

## Discussion

The results of the present study support the usefulness of the maternal supplementation with hydroxytyrosol to improve prenatal development in a swine model of IUGR pregnancies. Supplementation was associated with higher mean birth weight and decreased incidence of IUGR and therefore of low-birth-weight piglets. The positive effects of hydroxytyrosol administration remained during lactation, leading to higher body-weight at weaning, especially in larger litters. It also led to deviations in body composition and metabolic indices from control piglets that suggest greater growth potential and viability.

In the current study, hydroxytyrosol supplementation during mid-to-late gestation improved pregnancy outcomes. This suggests positive effects on the feto-placental unit, since the main cause of IUGR is believed to be deficiencies in placental development and efficiency (Wu et al., 2006; Vuguin, 2007). Hydroxytyrosol may improve placental function through its antioxidant and immuno-modulatory effects (Tundis et al., 2008; Rigacci and Stefani, 2016), since the placenta is tightly regulated by the immune-angiogenesis axis at the maternal interface (Kridli et al., 2016). Most of the data come from studies in adult humans and the information about their effects on pregnancy-related complications is still scarce (Ly et al., 2014). Our findings suggest that hydroxytyrosol and perhaps other polyphenols may exert a range of positive influences on pre- and post-natal development, but further specific studies for confirming our hypothesis are necessary.

Polyphenols intake increases plasma antioxidant capacity in humans (Prior et al., 2007; Ly et al., 2014) and, polyphenols supplementation has been found to improve placental oxidative stress, both *in vivo* and *in vitro* (Chen et al., 2012). This is of paramount importance for the development of preventive and therapeutic strategies aiming to favor the adequate development of the feto-placental unit, since it is well-known that an adequate antioxidant capacity in pregnant women is related, through improvement of oxidative stress status, to alleviation of the IUGR process (Burton et al., 2009; Myatt, 2010; Jauniaux and Burton, 2016). Studies linking polyphenols with health benefits have led to increased consumption of polyphenol-rich food and drinks among pregnant women, although further studies need to be undertaken (Granados-Principal et al., 2010; Ly et al., 2014; Parkinson and Cicerale, 2016) for establishing their realistic benefits, and even their potential hazards considering some evidence about adverse effects on fetal health

(Zielinsky and Busato, 2013) and epigenetic changes in offspring (Rigacci and Stefani, 2016).

In this scenario, the results obtained in the present study support the positive effects of hydroxytyrosol supplementation on adequate pregnancy development, through alleviation of the IUGR process and increase of mean birth-weight. In swine practice, birth-weight is determinant of piglet viability and survival; low birth-weight and within-litter birth-weight heterogeneity are the most important risk factors for perinatal morbidity and mortality (van der Lende et al., 2001; Yuan et al., 2015). These two problems can be particularly severe in large litters because of the competition over adequate uterine space for placental development (Ashworth et al., 2001; Foxcroft et al., 2006). In addition, the oncoming ADWG and Feed Conversion Rate (FCR) during the fattening period are strongly correlated with these traits (Powell and Aberle, 1980; Quiniou et al., 2002), becoming a topic of major importance due to the derived economic implications in swine production. A similar situation, in the largest litters, has been found in the present study with Iberian sows (a breed with a mean prolificacy around 6.5 piglets (Suárez MV, 2002)), since the newborns were heavier in non-prolific than in prolific litters (2-6 and 7-10 piglets, respectively). In spite of such difference, hydroxytyrosol was equally effective for increasing birth-weight in both prolific and non-prolific litters.

Afterwards, the benefits from hydroxytyrosol supplementation lasted during early postnatal stages, during the lactation period. At 15 days of age, mean body-weight remained higher in offspring from supplemented pregnancies than in controls; the effect was again found in piglets from both prolific and non-prolific litters, despite of fostering. Conversely, there was a significant effect of the litter size on body-weight between 15 and 25 days of age. At weaning, at 25 days of age, there were no differences in the body-weight of piglets from treated and control litters with 2-8 neonates. This result may reflect the higher ADWG of control piglets on days 15 and 25, which we are unable to explain and which deserves further study. On the other hand, hydroxytyrosol supplementation favored the ADWG and therefore, the growth of individuals from the most prolific litters (9-10 newborns). The consequence of a higher birth-weight plus a higher ADWG at weaning was a body-weight around 20% higher at weaning in these piglets than in control piglets.

We cannot elucidate, with the design of the current study, if these effects are directly related to a permanence of the effects from hydroxytyrosol supplementation, but we can

hypothesize that may be more likely an indirect effect caused by higher birth-weight since higher birth-weight by itself is associated with better post-natal growth performance (Rehfeldt and Kuhn, 2006). In any case, the benefits of maternal hydroxytyrosol supplementation for post-natal development are clear; in addition to increased body weight, supplementation was associated with greater content of subcutaneous fat and loin muscle. Prolificacy is an important trait in the pig production for its economic implications and any improvement is highly relevant. However, a higher prolificacy may be detrimental for offspring growth. Higher values of phenotypical characteristics (body-weight, back-fat depth, loin diameter) at weaning prove a good growth in the lactation period and are known to be related to a better development, and better yields, during the growing phase (Rehfeldt et al., 2012a; Rehfeldt et al., 2012b; Patience et al., 2015).

Independently of hydroxytyrosol supplementation, we observed a strong correlation between birth-weight and offspring sex, with males significantly heavier than females at birth. Nevertheless, both sexes attained similar body-weight during lactation. These data are consistent with results reported in previous work involving the same breed and dietary restriction (Gonzalez-Bulnes et al., 2012); together, these two studies support the idea that female piglets born to nutritionally restricted sows can show "catch-up growth" during lactation. We also observed evidence that piglets in the hydroxytyrosol group were better able to adapt to nutritional restriction than control neonates: We need to have in mind that catch-up growth of females during the early-postnatal period is a consequence of the prenatal restriction used in our model for increasing the incidence of IUGR processes. Hence, the findings of the current study may indicate a better adaptation to nutritional challenges of fetuses in the hydroxytyrosol group. These data are reinforced by stronger asymmetrical growth patterns in control piglets than in offspring from treated sows from favoring the development of brain, liver and other viscerae (main characteristics of nutritional IUGR; (Gonzalez-Bulnes et al., 2016)).

Hydroxytyrosol supplementation also showed significant effects on glucose and lipid metabolism in the offspring, primarily in piglets from larger litters. These piglets showed lower concentrations of fructosamine (a better index than glucose itself for long periods since represents average glucose values during previous days) and lower concentrations of total and LDL cholesterol. Decreases in these two parameters were also reported in humans with cardiometabolic disorders following intake of other polyphenols (Andreadou et al., 2006; Lin et al., 2009; Hu et al., 2014; Lockyer et al., 2016). In contrast to those human studies, hydroxytyrosol in our study was associated with higher, rather than

lower, plasma concentrations of triglycerides. These results make necessary the development of further studies but we may hypothesize that the increase of triglycerides found in the HT piglets of our study may be related to their higher back-fat depth (i.e.: a higher fat deposition).

In conclusion, supplementation of maternal diet with hydroxytyrosol during pregnancy improves pre- and early post-natal developmental patterns and metabolic traits of the offspring. The major benefits are increased birth-weight, and lower incidence of IUGR and therefore of low-birth-weight piglets. In addition, in piglets from the largest litters, supplementation is associated with higher ADWG and body-weight at weaning.

## Conflict of interest

There is no conflict of interest that would prejudice the information offered in the paper, excepting that AGB and CO are PLOS ONE Editorial Board members. However, this does not alter the authors' adherence to all the PLOS ONE policies on sharing data and materials.

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## Author Contributions

Conceptualization: MVG, SA, BI, AGB; Investigation: MVG, CGC, SA, LTR, JLP, EGF, RSS, PGA, BI, CO, AGB; Methodology: MVG, CGC, SA, LTR, JLP, EGF, RSS, PGA, BI, AGB; Formal analysis: MVG, CGC, SA, BI, AGB; Data curation: MVG, CGC, SA, BI, AGB; Writing (original

draft preparation): MVG, SA, BI, AGB; Writing (review and editing): CGC, LTR, EGF, RSS, PGA, JLP, CO; Project administration: AGB; Funding acquisition: AGB; Supervision: AGB.

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# 5. Discusión general

## 5.1. Efecto del peso al nacimiento en la producción del cerdo ibérico

La falta de homogeneidad en los lotes durante el crecimiento y en matadero es uno de los principales problemas para optimizar la rentabilidad de la producción porcina en razas genéticamente seleccionadas. Según la bibliografía, la variabilidad del PN es uno de los factores con mayor importancia en esta falta de homogeneidad (Wu *et al.* 2008; López-Vergé *et al.* 2018). Se trata de un problema importante puesto que un menor PN se asocia con una mayor mortalidad durante la lactación y con patrones alterados de desarrollo postnatal (Quiniou *et al.* 2002; Wu *et al.* 2006; Beaulieu *et al.* 2010; Jourquin *et al.* 2016). En razas genéticamente seleccionadas, la presencia de este tipo de lechones aumenta la variabilidad intracamada de PN, que se sitúa entre 15-24%, y se incrementa con un mayor tamaño de camada (Milligan *et al.* 2002; Quesnel *et al.* 2008).

En nuestro presente estudio en la raza ibérica, la variabilidad del PN estuvo en rangos similares, entre 12 y 22%, y también se observó un incremento en esta variabilidad y una disminución del PN medio al aumentarse la proporción de lechones de menor PN en las camadas de gran tamaño (10-13 lechones). Nuestros resultados indican que el aumento del tamaño de camada en cerdas ibéricas penaliza aún más el PN que en cerdas de genotipos con mayor prolificidad. En este sentido, se observan disminuciones de 43 g de PN al aumentar una unidad los nacidos totales en lechones ibéricos mientras las disminuciones son de 35 g en los lechones de razas más prolíficas (Quesnel et al. 2008; Beaulieu et al. 2010),

En nuestros resultados observamos que la restricción materna, cuando no incluye los últimos días de gestación (hasta el día 90), no afecta significativamente el PN de los lechones, aunque en estudios con restricciones al 50% hasta el parto se ha visto que el PN disminuye en los lechones restringidos (Gonzalez-Bulnes *et al.* 2012a; Barbero *et al.* 2013). La falta de efecto se debería al menor grado de restricción, pero principalmente a su diferente duración, ya que la restricción no incluyó la etapa de mayor demanda fetal al final de la gestación. Nuestros resultados coinciden con estudios en razas magras con restricciones moderadas (Bee 2004; Wu et al. 2006). No obstante, sí se detectaron efectos sobre las medidas morfológicas. Estas diferencias podrían llegar a utilizarse para identificar lechones con menor potencial de crecimiento, ya que Douglas et al. (2016) encontraron mayores correlaciones del rendimiento postnatal con los parámetros morfológicos que con el PN. Estos hallazgos sugieren la necesidad de estudios en

profundidad que permitan evaluar la utilidad de este tipo de medidas en condiciones de granja.

### 5.1.1. Efectos durante el desarrollo postnatal

En nuestro estudio, el crecimiento durante la lactación estuvo muy influido por el PN. Los mejores datos de crecimiento se observaron en lechones de mayores PN, con una diferencia de 70 g de GMDP entre los lechones de MBPN y los de APN. Por otro lado, la restricción materna durante la gestación, a pesar de no afectar el PN, sí mostro efectos sobre los índices de crecimiento durante la lactación y en etapas posteriores; así, el grupo de los lechones control de PNN tuvo el mayor crecimiento de todos los grupos. Al mismo tiempo, los lechones de BPN procedentes de madres restringidas presentaron una diferencia en el PV de 0.5 kg más al destete, y con mayor acumulación grasa, que los lechones de su misma categoría en el grupo control. Estos resultados podrían estar relacionados con cambios observados en la regulación del apetito y en rutas metabólicas relacionadas con la resistencia a la insulina en estudios previos sobre la RCIU (Cettour-Rose *et al.* 2005; Ovilo *et al.* 2014c).

Durante la fase de transición, los lechones de BPN presentaron un crecimiento compensatorio que se extendió durante el inicio de la fase de crecimiento. Posteriormente, el crecimiento fue similar en todos los grupos alcanzando todos ellos un PV y grosor de grasa subcutánea similares a los 110 días de vida. El crecimiento compensatorio fue más evidente en las hembras, sobre todo en las de menor PN. Además, en este periodo los cerdos provenientes de madres expuestas a la restricción materna alcanzaron un peso similar a los cerdos controles. Sin embargo, las hembras de menor PN de madres restringidas tardaron 40 días más en alcanzarlo, lo que refuerza el efecto negativo de la restricción materna sobre el desarrollo de la progenie. En estudios previos, el crecimiento compensatorio se describe como una estrategia encaminada a aumentar, la GMDP y la acumulación grasa tras una restricción de energía (Daza *et al.* 2003; Pugliese *et al.* 2005; Heyer and Lebret 2007). El crecimiento compensatorio observado en nuestros experimentos en los animales con menores PV podría ser un mecanismo para mejorar su supervivencia; como ya ha sido observado en el cerdo ibérico, concretamente en hembras (Gonzalez-Bulnes *et al.* 2012a; Barbero *et al.* 2013; Ovilo *et al.* 2014c).

Tras el crecimiento compensatorio, las hembras presentaron un menor crecimiento que fue especialmente negativo en las hembras de menor PN, que no consiguieron mantener el ritmo de desarrollo esperado hasta matadero (**Figura 5.1.**), independientemente de la nutrición de las madres durante la gestación. Este menor crecimiento de las hemnbras también se ha podido observar en otros estudios en razas magras (Latorre et al. 2003; Bérard et al. 2010).

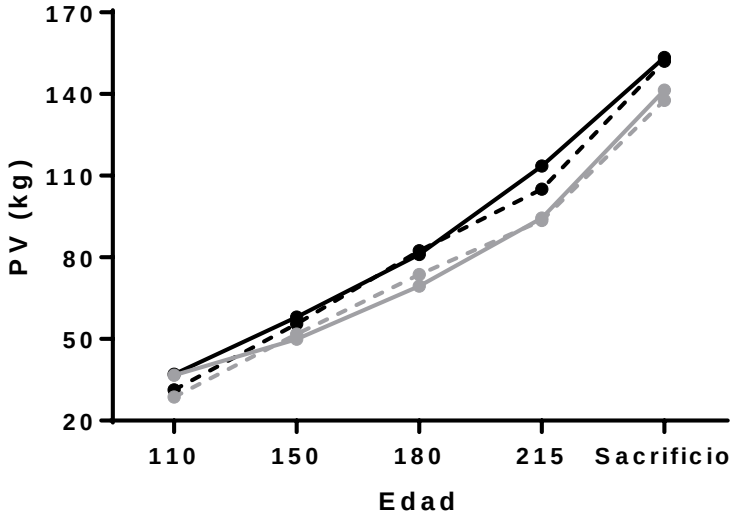


**Figura 5.1.** Evolución del peso vivo (PV; kg) en hembras desde el inicio de la fase de crecimiento hasta el matadero según su peso al nacimiento (PN) y la nutrición materna durante la gestación. Grupos: Control de PN normal (línea negra sólida), restringidas de PN normal (línea discontinua negra), control de bajo PN (línea gris sólida) y restringidas de bajo PN (línea discontinua gris),

Posteriormente, los cerdos con menor PN tuvieron un crecimiento más lento y, en general, menos eficiente durante la fase de cebo, aumentando los días de permanencia en la granja (**Figura 5.2.**), También los animales con restricción materna durante la gestación tuvieron un crecimiento más lento que los cerdos de las gestaciones controles, pero el efecto en la edad a matadero fue menor que en el caso del PN. De hecho, la influencia del PN sobre la edad al sacrificio parece ser mayor en razas grasas, con 15 días para los machos y 43 días para las hembras entre los grupos extremos de PN, que en razas magras, con 10 días de diferencia contando con ambos sexos (Beaulieu *et al.* 2010).

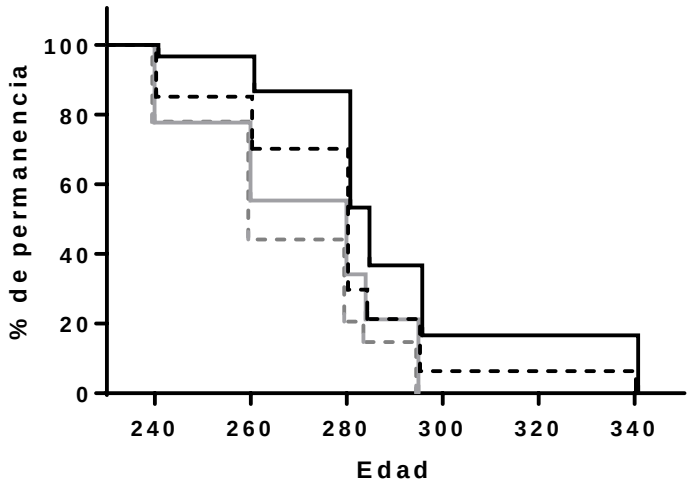


**Figura 5.2.** Porcentaje de cerdos que permanecieron en la en la granja tras el primer viaje a matadero según su grupo de peso al nacimiento (PN). Grupos: muy bajo PN (línea negra sólida), bajo PN (línea discontinua negra), PN medio (línea gris sólida) y alto PN (línea discontinua gris),

Este aumento de los días a matadero en el cerdo ibérico supone un incremento de los costes por animal y día en granja, es decir un aumento en días no productivos, concepto utilizado habitualmente para las reproductoras. En nuestras condiciones experimentales podemos cuantificar que animales con un PN menor a 1 kg y según el sexo generan un gasto adicional medio que oscila entre 17-39 Euros/cerda y 11-25 Euros/cerdo respecto a los cerdos de PN medio de la población (1.33 kg). Incluso, estos márgenes aumentarían 11 Euros en el caso de las cerdas y 7 Euros en el caso de los machos al compararlos con el límite inferior del grupo de mayor PN (1.54 kg). Además, hemos calculado la rentabilidad de los cerdos de menos de 1 kg de PN, teniendo en cuenta que todos los cerdos van a matadero al alcanzar el peso al sacrificio óptimo y que el cerdo ibérico de cebo se paga a unos 2 Euros/kg de PV (Salamanca 2018) y teniendo en cuenta también los consumos medios diarios durante la fase de crecimiento y cebo (desde el día 71 hasta matadero). Los cálculos muestran la menor rentabilidad de los cerdos de menos de 1 kg de PN, ya que sólo se obtiene un quinto de los beneficios netos que se consiguen con los cerdos de PN superior a 1.19 kg.

Por otro lado, el momento en el que se alcanza el peso a sacrificio es clave en la producción porcina. En el caso del cerdo ibérico la edad de sacrificio es un factor importante. En los resultados de los experimentos 1 y 2 puede observarse que casi todos los grupos, salvo las hembras de menor PN, tienen medias de edad inferiores a las

establecidas en la legislación (300 días), quedando la mayoría de las canales fuera de la norma. Los cerdos ibéricos normalmente son restringidos durante la fase de crecimiento y cebo, pero en nuestros experimentos se pretendió observar el potencial de crecimiento real de estos animales. En nuestros estudios, se puede ver que el uso de la restricción prenatal en los cerdos con menores PN, especialmente aquellos menores de 1 kg, podría comprometer su crecimiento aumentando su permanencia en la granja y perjudicando la rentabilidad del lote. En un futuro, se deberían estudiar las posibles estrategias a utilizar para manejar la variabilidad de los lotes teniendo en cuenta el PN, especialmente para los cerdos de menos de 1 kg.

## 5.1.2. Efectos a nivel metabólico y fisiológico

Las diferencias en los patrones de crecimiento postnatales entre los grupos de PN y entre los cerdos con diferentes niveles de nutrición materna durante la gestación sugieren la existencia de diferencias en los patrones metabólicos de los diferentes grupos, Se han observado diferencias en los patrones de desarrollo muscular y de acumulación de grasa entre los cerdos de menor y mayor PN y entre los cerdos procedentes de gestaciones con restricción alimentaria y de gestaciones control. Un caso especial a destacar son las hembras de menos de 1 kg de PN, ya que su acumulación grasa, al igual que su crecimiento durante las fases de crecimiento y cebo, fue menor que en el resto de los grupos, lo que dificulta la interpretación de los datos en los cerdos de menor PN.

En relación al desarrollo muscular, por un lado, los lechones con el menor PN y los procedentes de madres restringidas tuvieron menor diámetro medio de LD al destete lo que puede estar asociado con la RCIU durante la fase de desarrollo de las miofibrillas secundarias que ocurre entre los días 55 y 90 de gestación y está modulado por factores nutricionales y epigenéticos (Dwyer and Stickland 1991; Dwyer *et al.* 1994; Picard *et al.* 2002). Sin embargo, no se encontraron diferencias el desarrollo muscular en la fase de cebo, lo que puede indicar una hipertrofia de las miofibrillas durante el crecimiento posnatal similar a la descrita en las razas más seleccionadas (Rehfeldt and Kuhn 2006; Rekiel et al. 2015). No obstante, esta hipótesis no puede comprobarse con nuestro diseño, por lo que se necesitarán estudios sobre las fibras musculares.

En cuanto a la acumulación grasa, a excepción de las hembras de MBPN, los cerdos de menor PN tienen una mayor tendencia a acumular grasa durante la fase de

cebo; especialmente en la capa interna de la grasa subcutánea que es la más activa metabólicamente (Hausman and Thomas 1984). En el caso de los machos la diferencia es de 0.3 cm de grosor entre el grupo de menor PN y el de mayor en la capa interna de la grasa subcutánea. Esta tendencia también ocurre en los cerdos de madres restringidas durante la gestación. Además, los marcadores del metabolismo en plasma de los cerdos de MBPN al final de su vida mostraron alteraciones en la glucosa y la secreción de insulina, modulada por el sexo, aunque sus niveles de acumulación de grasa fueron similares al sacrificio. En el caso de los cerdos procedentes de madres restringidas, se encontraron indicadores de dislipemia que evidencian una mayor tendencia a la resistencia a la insulina y una mayor deposición de grasa durante la fase de cebo (Cettour-Rose et al. 2005; Berends et al. 2013).

Por otra parte, el análisis de AG del hígado refuerza la hipótesis de alteraciones metabólicas y de posibles trastornos metabólicos como excesivo engrasamiento (obesidad) o la resistencia a la insulina en cerdos de menor PN y procedentes de madres restringidas durante la gestación en ambos sexos (Attie et al. 2002; Hulver et al. 2005; Poudyal and Brown 2011). En los cerdos de gestaciones con restricción alimentaria se encontraron mayores índices de desaturación que en los cerdos de gestaciones controles. Estos resultados están relacionados con una mayor actividad de la enzima estearoil-CoA desaturasa-1 (SCD1) que a su vez se asocia con el aumento de la lipogénesis y la acumulación de grasa (Paillard et al. 2008). En los cerdos con los menores PN, también se observaron estos resultados; incluso en los cerdos de un PN inferior a 1.2 kg que no mostraron alteraciones a nivel plasmático. Por otro lado, las hembras menores de 1 kg de PN tuvieron bajos índices de desaturación de los AG lo que se promueve la oxidación de lipídica y podría estar ligado a su menor acumulación grasa en el hígado (Dobrzyn et al. 2004; Dobrzyn et al. 2005).

Las posibles alteraciones metabólicas observadas durante el cebo, en cerdos de menor PN y los afectados por la restricción materna durante la gestación, podrían empeorar con el paso del tiempo; aunque esta hipótesis no puede ser comprobada con el diseño del presente estudio y serían necesarios más estudios para profundizar en ella. Por un lado, sería necesario comprender con mayor exactitud los procesos metabólicos alterados en este tipo de animales, mediante, por ejemplo, el análisis de su transcriptoma, metaboloma y epigenoma. Y por otro, hay que reflexionar sobre el uso de este tipo de animales como reproductores, en especial las hembras, ya que podría conducir a alteraciones transgeneracionales que condicionen el desarrollo de sus progenies.

## 5.1.3. Efectos en la calidad de la canal y de la carne

Los diferentes patrones de crecimiento y metabolismo pueden afectar a la calidad de la canal y de la carne al sacrificio. Respecto a la calidad de la canal, los cerdos de menor PN no mostraron diferencias en el rendimiento de la canal, pero sí mostraron canales 2.5 cm más cortas que los cerdos de mayor PN, lo que podría estar relacionado con su peor desarrollo en la fase de engorde. Este efecto, conocido en las razas magras, se asocia a un menor peso de las piezas nobles, menor contenido de carne y peor calidad (Rekiel *et al.* 2014). Además, los cerdos procedentes de madres sometidas a restricción durante la gestación tuvieron menores rendimientos de la canal lo que podría relacionarse con un menor desarrollo muscular o, por otro lado, con una mayor cantidad de grasa visceral o un mayor desarrollo de los órganos, ya observados en estudios anteriores (Barbero *et al.* 2013; Ovilo *et al.* 2014c).

Aunque los cerdos de menor PN tienen una mayor tendencia a acumular grasa, no se encontraron diferencias en el grosor de grasa subcutánea entre los grupos de PN al sacrificio. Este hecho puede deberse a la fisiología del cerdo ibérico, concretamente a su elevada capacidad de engrasamiento, puesto que sí se han observado diferencias en razas magras (Bee 2004; Gondret *et al.* 2006; Bérard *et al.* 2010). Sin embargo, los cerdos procedentes de gestaciones con restricción alimentaria, que también tuvieron una mayor tendencia a la acumulación de grasa en el crecimiento, presentaron un menor grosor de grasa subcutánea al sacrificio. Estos datos sugerirían que los cerdos con BPN y los cerdos procedentes de madres restringidas durante la gestación tienen diferentes patrones metabólicos en la acumulación de grasa durante la fase de cebo.

Entre los parámetros que se utilizan para medir la calidad de la carne, el valor de la GIM es de gran importancia. En nuestro estudio, los cerdos de menor PN tuvieron una mayor cantidad de GIM, lo que podría estar relacionada con la hiperplasia de adipocitos a nivel fetal en concordancia con la hipótesis de la programación prenatal (Poore and Fowden 2004; Hausman *et al.* 2014; Gonzalez-Bulnes *et al.* 2016a). Sin embargo, los cerdos procedentes de madres restringidas durante la gestación tuvieron valores similares a los controles, reforzando la hipótesis de la existencia de diferencias metabólicas o adaptativas a nivel de la acumulación de grasa entre cerdos procedentes de gestaciones control y de gestaciones con restricción alimentaria.

En los datos relacionados con la calidad de la carne, también se han encontrado diferencias en la composición de los AG. En la bibliografía, los datos del efecto del PN sobre la composición de los AG de los tejidos son escasos. Los estudios realizados en esta tesis doctoral son los primeros que analizan dicha composición en el hígado, en la grasa subcutánea, y en las fracciones de lípidos mayoritarias de la GIM (FLN y FLP). En la literatura previa, Alvarenga et al. (2014) no detectaron ningún efecto del PN en la composición de la GIM; sin embargo en nuestro estudio se encontraron efectos del PN en dicha composición aunque diferentes a las encontrados por Rekiel *et al.* (2014) y a los encontrados en el hígado. Este hecho es concordante con las diferencias en el metabolismo de AG entre tejidos (Sampels et al. 2011).

La valoración de FLN de la GIM muestra que los cerdos de PN mayor a 1 kg, y especialmente los grupos más pesados, presentan mayores niveles de AG poliinsaturados y monoinsaturados. Estas características son comercialmente interesantes ya que podrían mejorar su aceptación por parte del consumidor debido a sus mejores características sensoriales y sus posibles beneficios para la salud (Laitinen et al. 2006; Jakobsen et al. 2009). Además, hemos encontrado un mayor contenido en C18: 1n-9 en los cerdos de mayor PN. Este AG se ha utilizado siempre como un indicador de calidad en los productos cárnicos porque se asocia con características organolépticas apreciadas por los consumidores y es el más abundante en el cerdo ibérico de montanera, que es el de mayor calidad de carne y relevancia económica (Rey *et al.* 1997; Barea *et al.* 2013). Por otro lado, la descendencia de cerdas con restricción durante la gestación tuvo más diferencias en la FLP que en la FLN, lo que podría estar relacionado con la lipooxidación o la movilización selectiva al considerarse la composición de los lípidos de membrana bastante estable (Herzberg and Farrell 2003; Sampels *et al.* 2011; Price and Valencak 2012).

La grasa subcutánea también es importante para de la calidad de carne especialmente en los productos curados; sin embargo, el PN no tuvo un gran efecto en el perfil de AG de la grasa subcutánea. La mayor parte de las diferencias encontradas fueron debidas a la nutrición materna durante la gestación y al sexo. Los animales procedentes de gestaciones con restricción alimentaria tenían un índice de desaturación más alto que los controles, lo que apoyaría las diferencias metabólicas encontradas en la fase de cebo entre estos dos grupos. Además, estos cerdos mostraron mayores concentraciones de C18: 2 n-6 en la capa externa, lo que puede llegar a perjudicar la producción de productos cárnicos de calidad ya que altas concentraciones de este AG están asociadas con

problemas de enranciamiento y de la migración de agua (Isabel et al. 2014). Por último, la existencia de diferencias debidas al sexo en el perfil de AG se ha observado en varios estudios (Segura et al. 2015; Daza et al. 2016; Egea et al. 2016). En nuestro caso, el sexo afectó por igual a la GIM y la grasa subcutánea. Así, los machos presentaron unos valores más acordes con las recomendaciones para dietas en humanos, que aconsejan una relación Σn-6 / Σn-3 cercana a 4/1 y mayores valores de AG monoinsaturados y poliinsaturados, que las hembras (Simopoulos 2002).

En el cerdo ibérico, el efecto del PN es mayor al principio de su vida y en la etapa final de cebo que durante la fase de crecimiento. Con los datos obtenidos sabemos que un menor PN, especialmente menor a 1.2 kg, incrementa los días a matadero. Sin embargo, al mismo tiempo las alteraciones en la calidad de la canal y de la carne por el PN son menores a las esperadas. Este menor efecto puede estar relacionado con el hecho de que los cerdos de menor PN se mataron con más edad puesto que a los 279 días, en todos los grupos de peso excepto el de mayor PN, quedaban por sacrificar más del 55% de los cerdos y a los 294 días más del 21%. Los resultados de esta tesis doctoral pueden ser de gran utilidad a nivel industrial en el cerdo ibérico puesto que son los primeros en describir el efecto del PN en la variabilidad de los lotes productivos.

Por otro lado, una ligera restricción de la alimentación materna antes del máximo crecimiento exponencial del feto puede parecer una buena estrategia a primera vista para ahorrar costes, al no observarse grandes diferencias al nacimiento o al destete. Sin embargo, más tarde la progenie presenta un peor crecimiento con alteraciones metabólicas y una peor calidad de la canal y de la carne respecto a los animales procedentes de gestaciones control, lo que puede penalizar la rentabilidad de la explotación.

## 5.2. Efecto de la suplementación prenatal de hidroxitirosol en la descendencia.

La variabilidad de PN, como se ha observado en los experimentos 1 y 2, puede tener efectos negativos sobre distintos aspectos productivos del cerdo ibérico. Estos efectos son especialmente graves en el caso de los cerdos de menor PN, que son los animales que presentan peores resultados en los parámetros de crecimiento postnatal y en las características de calidad de la canal y de la carne. Por ello, el uso de estrategias nutricionales durante la gestación es aconsejable para disminuir la variabilidad del PN y los lechones de BPN. Con ellas se pretende la reducción de los efectos negativos de la RCIU, como por ejemplo un mayor estrés oxidativo que puede desencadenar inflamación de grado bajo.

En los últimos años, se ha incrementado el interés en el uso de polifenoles, principalmente por sus propiedades antioxidantes, y antiinflamatorias, como estrategia nutricional para mejorar el desarrollo fetal (Gonzalez-Bulnes *et al.* 2018). Entre los polifenoles con mayor potencia antioxidante se encuentra el HT y, además, no tiene efectos tóxicos conocidos. Por ello, en el experimento 3 se evaluó la utilidad de la suplementación materna con HT.

Los resultados muestran que la suplementación materna con HT durante los dos últimos tercios de gestación mejora el desarrollo fetal en la raza ibérica, al relacionarse con un mayor PN medio y un menor índice de lechones de BPN (Wu *et al.* 2008; Vallet *et al.* 2014; Rigacci and Stefani 2016). En cualquier caso, estos resultados son muy prometedores pero deben considerarse como un primer paso previo a estudios más detallados que evalúen los cambios que se producen a nivel placentario y fetal para una mejor comprensión de los efectos del HT durante la gestación. Por otro lado, también es necesaria la realización de otros estudios que permitan definir la dosis efectiva de HT.

El efecto positivo de la suplementación materna con HT se extiende durante la lactación, presentando los lechones del grupo HT un mayor PV al destete. Los lechones del grupo HT también mostraron un menor crecimiento asimétrico al destete en las visceras al no favorecer el desarrollo de cerebro, hígado y otras vísceras, hecho que fue mayor en los lechones del grupo C y que se relaciona con la RCIU (Wu et al. 2006; Gonzalez-Bulnes et al. 2016b). Aun así, no podemos afirmar que el efecto observado durante la lactación de los lechones del grupo HT sea un efecto directo de la suplementación durante la gestación. Ya que podría tratarse de un efecto indirecto por un

mayor PN, que como hemos podido comprobar en el experimento 1 está asociado con un mejor desarrollo postnatal. Sin embargo, el crecimiento en torno a un 20% superior de los lechones del grupo HT de las camadas de mayor tamaño al nacimiento (9-10 lechones) respecto a sus pares del grupo C, podría indicar un efecto a medio plazo de la suplementación en gestación de HT en las camadas que más sufren la RCIU.

Además, independientemente de la suplementación con HT, se observó nuevamente la influencia del sexo confirmándose que las hembras nacidas de gestaciones con restricción alimentaria pueden tener un crecimiento compensatorio durante la lactación (**Tabla 5.1.**; Gonzalez-Bulnes *et al.* 2012a; Barbero *et al.* 2013; Ovilo *et al.* 2014c).

**Tabla 5.1.** Pesos al nacimiento y al destete (kg) de hembras y machos, independientemente del tratamiento con hidroxitirosol. **18**

| | Hembras | Machos | valor *P* |
|---|---|---|---|
| Nacimiento | 1.15 ± 0.03 | 1.25 ± 0.02 | <0.0005 |
| Destete | 5.03 ± 0.14 | 5.17 ± 0.11 | ns |

Media ± Error estándar de la media. Ns= no significativo

Aunque los resultados muestran que la suplementación prenatal con HT tiene efectos beneficiosos, es necesario realizar más experimentos para evaluar las posibles consecuencias durante el resto del ciclo productivo y confirmar la posibilidad del uso del HT como suplemento en piensos de gestación, además de conocer sus mecanismos de acción. Por otro lado, los experimentos sobre el uso de HT en porcino pueden tener un doble interés al considerarse al cerdo un buen modelo animal para estudios en biomedicina, ya que en humanos la RCIU aumenta el riesgo de sufrir complicaciones postnatales y enfermedades no transmisibles "programadas" a nivel prenatal como la obesidad, la diabetes o enfermedades cardiovasculares, (Barker 1998; McMullen and Mostyn 2009; Prather 2013). Entre los posibles modelos animales, destaca el cerdo ibérico, como modelo animal no transgénico que puede ser usado para el estudio de los efectos de la obesidad o la resistencia a la insulina en la gestación y sus efectos en la progenie (Spurlock and Gabler 2008; Gonzalez-Bulnes *et al.* 2016a).

# Conclusiones

1. El aumento de la prolificidad en el cerdo ibérico incrementa la cantidad de lechones de bajo peso al nacimiento y la variabilidad intracamada del peso al nacimiento. Además, un mayor tamaño de camada produce una mayor disminución en el peso al nacimiento de los lechones en la raza ibérica que en las razas genéticamente seleccionadas.

2. Al igual que en otras razas, en la raza ibérica un menor peso al nacimiento se relaciona con un crecimiento postnatal más lento, menos eficiente y con alteraciones metabólicas. Algunos de estos efectos están modulados por el sexo, e incluso son más drásticos que los descritos en razas magras, causando un incremento de la edad a matadero y una disminución de la calidad de la canal y de la carne.

3. Una restricción nutricional materna moderada durante el segundo y parte del tercer tercio de gestación en el cerdo ibérico tiene efectos negativos en el crecimiento postnatal y la calidad de la canal y de la carne, a pesar de no haber efectos negativos en el peso al nacimiento.

4. En el cerdo ibérico en condiciones de granja, los datos obtenidos sugieren establecer el valor de 1 kg de peso al nacimiento para el triaje de los lechones de bajo peso al nacimiento para usar futuras estrategias nutricionales y de manejo.

5. La suplementación materna con hidroxitirosol de 1.5 mg/día durante la gestación mejora el desarrollo prenatal, con mayores pesos al nacimiento y una menor incidencia de lechones inferiores a 1 kg de peso al nacimiento, así como el desarrollo postnatal temprano de la descendencia.

6. Los efectos de la suplementación materna con hidroxitirosol son especialmente beneficiosos en las camadas de mayor número de lechones favoreciendo un mayor crecimiento durante la lactación.

# Conclusions

1. The increase in prolificacy in the Iberian breed increases the number of low birth weight piglets and the within-litter variability of birth weight. In addition, a larger litter size produces a greater decrease in the birth weight of Iberian piglets than of that of piglets from genetically selected breeds.

2. Like in other pig breeds, in the Iberian breed a lower birth weight is related to slower and less efficient postnatal growth and metabolic alterations. Some of these effects are modulated by sex, and are even more drastic than those described in lean breeds, causing an increase in slaughter age and a decrease in the carcass and meat quality.

3. A moderate maternal nutritional restriction during the second and part of the last third of gestation in the Iberian pig has negative effects on the postnatal growth and the carcass and meat quality, despite no significant effects on birth weight.

4. In the Iberian pig under farm conditions, the data obtained suggest establishing the value of 1 kg of birth weight for the triage of low birth weight piglets to consider future nutritional and management strategies.

5. Maternal supplementation of hydroxytyrosol with 1.5 mg/day during pregnancy improves prenatal development of the offspring, with higher birth weights and a lower incidence of piglets under 1 kg of birth weight, as well as early postnatal development.

6. The effects of maternal supplementation with hydroxytyrosol are especially beneficial for the largest litters, favoring greater growth during lactation.

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# Anexo 1: Otras publicaciones y comunicaciones en congresos durante la tesis doctoral

## Publicaciones indexadas

1. **Vázquez-Gómez, M.**, García-Contreras, C., Torres-Rovira, L., Astiz, S.,... & Isabel, B. 2018. Maternal undernutrition and offspring sex determine birth-weight, postnatal development and meat characteristics in traditional swine breeds. Journal of Animal Science and Biotechnology. doi: 10.3390/ijms19010232

2. Pesantez, JL., Torres-Rovira, L.,... **Vázquez-Gómez, M.**,... & Astiz, S. 2018. Efficiency and demographics of a high-yield dairy ewe farm with two managing systems involving five or 10 lambings per year. Animal. doi: 10.1017/S175173111700369X

3. García-Contreras, C., **Vázquez-Gómez, M.**, Torres-Rovira, L., Gónzalez, J.,... & González-Bulnes, A. 2018. Characterization of Ageing- and Diet-Related Swine Models of Sarcopenia and Sarcopenic Obesity. International Journal of Molecular Sciences. doi: 10.3390/ijms19030823

4. Luis-Lima, S., García-Contreras, C., **Vázquez-Gómez, M.**, Astiz, S.,... & Porrini, E. 2018. A Simple Method to Measure Renal Function in Swine by the Plasma Clearance of Iohexol. International Journal of Molecular Sciences. doi: 10.1186/s40104-018-0240-6

5. García-Contreras, C., **Vázquez-Gómez, M.**, Astiz, S., Torres-Rovira, L.,... & González-Bulnes, A. 2017. Ontogeny of Sex-Related Differences in Foetal Developmental Features, Lipid Availability and Fatty Acid Composition. International Journal of Molecular Sciences. 2017(18):1171. doi: 10.3390/ijms18061171

6. **Vázquez-Gómez, M.**, García-Contreras, C., Torres-Rovira, L., Pesantez, JL.,... & González-Bulnes, A. 2017. Polyphenols and IUGR pregnancies: Maternal hydroxytyrosol supplementation improves prenatal and early-postnatal growth and metabolism of the offspring. PloS ONE. 12(5):e0177593. doi: 10.1371/journal.pone.0177593

7. García-Contreras, C., Valent, D., **Vázquez-Gómez, M.**, Arroyo, L., Isabel, B.,... & González-Bulnes, A. 2017. Fetal growth-retardation and brain-sparing by malnutrition are associated to changes in neurotransmitters profile. International journal of developmental neuroscience. doi: 10.1016/j.ijdevneu.2017.01.005

8. **Vázquez-Gómez, M.**, Valent, D., García-Contreras, C., Arroyo, L., Óvilo, C., Isabel, B.... & González-Bulnes, A. 2016. Sex and intrauterine growth restriction modify brain neurotransmitters profile of newborn piglets. International journal of developmental neuroscience. 55. doi: 10.1016/j.ijdevneu.2016.09.004

9. Gonzalez-Bulnes, A., Astiz, S., Óvilo, C., Parraguez, V. H., Garcia-Contreras, C., & **Vázquez-Gomez, M**. 2016. Empowering translational research in fetal growth restriction: Sheep and swine animal models. Current pharmaceutical biotechnology. doi: 10.2174/1389201017666160519111529

10. Gonzalez-Bulnes, A., Astiz, S., Óvilo, C., Garcia-Contreras, C., & **Vázquez-Gomez, M**. 2016. Nature and Nurture in the Early-Life Origins of Metabolic Syndrome. Current pharmaceutical biotechnology. 17(7): 573-86. doi: 10.2174/1389201017666160301103835

11. Gonzalez-Bulnes, A., Astiz, S., Óvilo, C.… & **Vázquez-Gómez, M**. Developmental Origins of Health and Disease in swine: implications for animal production and biomedical research. Theriogenology. 86 (1) .doi: 10.1016/j.theriogenology.2016.03.024

12. Gonzalez-Bulnes, A., Astiz, S., **Vázquez-Gómez, M.**, & Garcia-Contreras, C. 2016. Developmental origins of metabolic disorders: The need for biomarker candidates and therapeutic targets from adequate preclinical models. EuPA Open Proteomics. 10: 50-55. doi: 10.1016/j.euprot.2016.01.001

13. Cogollos, L., Garcia-Contreras, C., **Vázquez-Gómez, M.**, Astiz, S., Sanchez-Sanchez, R., Gomez-Fidalgo, E.,… & Gonzalez-Bulnes, A. 2016. Effects of fetal genotype and sex on developmental response to maternal malnutrition. Reproduction, Fertility and Development. doi: 10.1071/RD15385

14. Gonzalez-Bulnes, A., Torres-Rovira, L., Astiz, S., Óvilo, C., Sanchez-Sanchez, R., Gomez-Fidalgo, E.,… & **Vázquez-Gómez, M**. 2015. Fetal Sex Modulates Developmental Response to Maternal Malnutrition. PloS ONE. 10 (11). doi: 10.1371/journal.pone.0142158.

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1. **Vázquez-Gómez, M.**, García-Contreras, C., Óvilo, C., González-Bulnes, A. & Isabel, B. 2018. Effects of sex and intrauterine growth restriction on fatty acid composition of Iberian newborn piglets. Archivos de Zootecnia. ISSN: 0004-0592.

2. Gonzalez-Bulnes, A., Astiz, S.,… **Vázquez-Gomez, M.**, & Garcia-Contreras, C. 2018. Implications of prenatal programming in Iberian pig production. Archivos de Zootecnia. ISSN: 0004-0592.

3. García-Contreras, C., **Vázquez-Gómez, M.,** Torres-Rovira, L., Astiz, S.... & González-Bulnes, A. 2018. Effect of sex and age on fatty acid composition of Iberian swine foetuses exposed to maternal malnutrition. Archivos de Zootecnia. ISSN: 0004-0592.

4. **Vázquez-Gómez, M.**, García-Contreras, C., Torres-Rovira, L., Isabel, B. & González, A. 2016. Factores prenatales y postnatales que influyen en el tipo de fibra muscular y la calidad de carne en el cerdo Ibérico. Sólo Cerdo Ibérico. 36. ISSN: 2254-4240.

5. García-Contreras, C., **Vázquez-Gómez, M.**, Torres-Rovira, L., Óvilo, C. & González, A. 2016. Factores ambientales y epigenéticos en la producción de cerdas hiperprolíficas. Avances en tecnología porcina. 13 (127). ISSN: 1697-2015.

6. Torres, L., **Vázquez, M.**, García, C. & González, A. 2016. Factores limitantes y posibles vías de mejora de la prolificidad y el peso al nacimiento de los lechones en cerdas ibéricas. Avances en tecnología porcina. 13 (126). ISSN: 1697-2015.

## Capítulos de libros

1. Gonzalez-Bulnes, A., Astiz, S., Isabel, B., **Vázquez-Gomez, M.**, & Garcia-Contreras, C. 2018. "Possible Benefits and Risks of Polyphenols Supplementation during Pregnancy". Polyphenols in Human Health and Disease. (19):249-260. ISBN: 9780128130070.

## Comunicaciones en congresos nacionales e internacionales

(persona que presenta; ^Presentación oral; *Poster)

1. **Vázquez-Gómez, M.***, Garcia-Contreras, C., Astiz, S.,... & Isabel, B. 2018. The effect of birth-weight on growth performance and meat quality in Iberian pigs. 66th EAAP Annual Meeting. Croacia.

2. Garcia-Contreras, C.*, **Vázquez-Gómez, M**., Torres-Rovira, L.,... & Gonzalez-Bulnes, A. 2018. Polyphenols and IUGR pregnancies: maternal hydroxytyrosol supplementation and foetal developmentrowth performance and meat quality in Iberian pigs. 66th EAAP Annual Meeting. Croacia.

3. Pesantez, JL.... **Vázquez-Gómez, M**, Garcia-Contreras, C.*.... & Astiz, S. 2018. Effects of maternal factors during pregnancy on the birth weight of lambs in dairy sheep. 66th EAAP Annual Meeting. Croacia.

4. Feyjoo, P., Pesantez, JL.,… **Vázquez-Gómez, M.***,…. & Astiz, S. 2018. Ten years evolution of dairy cattle herds: fertility, production and management. 66th EAAP Annual Meeting. Croacia.

5. Boudon, A.*, Conde-Aguilera, A.,… **Vázquez-Gómez, M.**,…. & Dourmad, JY. 2018. Effet de la supplémentation en magnésium sur son utilisation digestive par le porc en croissance nourri avec des régimes à teneurs en fibres variables. 50èmes Journées de la Recherche Porcine. Francia.

6. **Vázquez-Gómez, M.†**, Garcia-Contreras, C., Torres-Rovira, L.,… & González-Bulnes, A. 2017. Effects of nutritional pregnancy management on carcass and meat features of offspring: Implications for the production of dry-cured products. 4th Fatty Pigs Conference. España.

7. Sanz-Fernandez, MV.^, Torres-Rovira, L., Pesantez, JL., **Vázquez-Gómez, M.**,… & Gonzalez-Bulnes, A. 2017. Effects of low birth weight on the immune function of Iberian piglets. 4th Fatty Pigs Conference. España.

8. **Vázquez-Gómez, M.^**, Garcia-Contreras, C., Óvilo, C., Gonzalez-Bulnes, A. & Isabel, B. 2017. Prenatal nutritional programming changes the fatty acid composition of offspring. Final Meeting of SALAAM Cost. Alemania.

9. Sanz-Fernandez, MV.^, Torres-Rovira, L., Pesantez, JL., **Vázquez-Gómez, M.**,… Gonzalez-Bulnes, A. & Gonzalez-Bulnes, A. 2017. Characterization of the Iberian pig as a model of obesity-induced changes in immune function. Final Meeting of SALAAM Cost. Alemania.

10. **Vázquez-Gómez, M.*,** Garcia-Contreras, C., Benítez, R., … & Óvilo, C. 2017. Altered gene expression of the appetite regulation pathway in low-birth-weight piglets. 36th ISAG Conference. Irlanda.

11. García-Contreras, C.*, Madsen, O., **Vazquez-Gómez, M.**,… & Óvilo, C. 2017. Effect of fetal genotype and weight on muscle transcriptome in Iberian pigs. 36th ISAG Conference. Irlanda.

12. **Vázquez-Gómez, M.**, Garcia-Contreras, C., Torres Rovira L.,… Astiz, S., Gonzalez-Bulnes, A.^. 2017. Polyphenol supplementation during pregnancy improves prenatal and early-postnatal growth and metabolism of the offspring. 11th World Congress on Polyphenols Applications: Vienna Polyphenols 2017. Austria.

13. García-Contreras, C.^, **Vazquez-Gómez, M.**, Astiz, S.,… & Óvilo, C. 2017. Efecto del genotipo y el peso fetal sobre el transcriptoma muscular del cerdo ibérico. XVII Jornadas sobre Producción Animal. España.

14. **Vázquez-Gómez, M.^**, García-Contreras, C., Segura, J.,…. & Isabel, B. 2017. Efecto de la restricción de crecimiento intrauterino y el sexo en el desarrollo postnatal en el cerdo ibérico. XVII Jornadas sobre Producción Animal. España.

15. Garcia-Contreras, C.*, **Vázquez-Gómez, M.**, Astiz, S,…. B. & Gonzalez-Bulnes, A. 2016. Effect of sex and age on fatty acids composition in Iberian swine fetuses exposed to maternal malnutrition. IX International Symposium on Mediterranean Pig. Portugal.

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# Anexo 2: Material suplementario Experimento 1

**Supplementary Table 1.** Calculated analysis (g/kg, dry-matter basis) and fatty acid composition of the diets.

| | Sow | | Offspring | | | | | |
|---|---|---|---|---|---|---|---|---|
| Item | Gestation | Lactation | 1st Prestarter | Prestarter | Starter | Growth 1 | Growth 2 | Finisher |
| Age | | | 7-25 d-old | 26-35 d-old | 36-70 d-old | 71-140 d-old | 141-210 d-old | from 211 d-old |
| Calculated analysis [1] | | | | | | | | |
| Net energy, Mcal $kg^{-1}$ | 2217.0 | 2250.0 | 2300.0 | 2400.0 | 2400.0 | 2350.0 | 2400.0 | 2464.0 |
| DM | 899.9 | 894.9 | 894.5 | 895.4 | 895.6 | 894.5 | 899.4 | 906.1 |
| Crude protein | 140.0 | 160.0 | 150.0 | 157.8 | 165.0 | 156.0 | 142.5 | 125.0 |
| Crude fat | 26.1 | 36.6 | 40.0 | 40.0 | 40.0 | 31.5 | 42.0 | 57.0 |
| Crude fiber | 64.0 | 50.0 | 20.0 | 29.3 | 28.8 | 35.0 | 35.0 | 34.0 |
| Nitrogen-free extracts | 606.7 | 584.6 | 634.5 | 621.6 | 613.9 | 622.4 | 631.6 | 643.3 |
| Ash | 63.1 | 63.7 | 50.0 | 46.7 | 47.9 | 49.6 | 48.3 | 46.8 |
| Fatty acids composition (g/100 g total FA) | | | | | | | | |
| C14:0 | 1.12 | 0.80 | 0.73 | 0.73 | 0.78 | 1.04 | 0.66 | 0.91 |
| C16:0 | 16.14 | 14.07 | 23.61 | 20.69 | 20.11 | 21.86 | 22.85 | 22.24 |
| C16:1 n-9 | 0.04 | 0.03 | 0.11 | 0.18 | 0.21 | 0.31 | 0.18 | 0.16 |
| C16:1 n-7 | 1.14 | 0.78 | 0.72 | 0.91 | 1.20 | 1.64 | 0.73 | 1.19 |
| C17:0 | 0.41 | 0.43 | 0.43 | 0.61 | 0.52 | 0.52 | 0.54 | 0.48 |
| C17:1 | 0.18 | 0.06 | 0.13 | 0.18 | 0.23 | 0.29 | 0.15 | 0.20 |
| C18:0 | 5.78 | 3.42 | 5.86 | 5.18 | 6.02 | 7.70 | 4.61 | 7.07 |
| C18:1 n-9 | 25.87 | 22.17 | 28.97 | 24.91 | 29.96 | 33.50 | 33.91 | 32.42 |
| C18:1 n-7 | 1.12 | 1.10 | 0.85 | 1.30 | 1.40 | 1.21 | 1.51 | 1.39 |
| C18:2 n-6 | 40.06 | 47.23 | 34.43 | 39.74 | 34.79 | 27.17 | 26.54 | 28.58 |
| C18:3 n-3 | 4.54 | 5.06 | 2.54 | 3.32 | 2.81 | 2.47 | 4.38 | 3.15 |
| C20:0 | 0.31 | 0.33 | 0.32 | 0.25 | 0.24 | 0.18 | 0.31 | 0.33 |
| C20:1 n-9 | 0.47 | 0.40 | 0.50 | 0.70 | 0.72 | 0.84 | 0.89 | 1.01 |
| C20:3n-6 | 0.16 | 0.13 | 0.11 | 0.09 | 0.13 | 0.17 | 0.06 | 0.11 |
| C20:4 n-6 | 0.02 | 0.11 | 0.04 | 0.26 | 0.05 | 0.08 | 0.19 | 0.05 |
| C20:5 n-3 | 0.91 | 1.34 | 0.02 | 0.25 | 0.19 | 0.10 | 0.15 | 0.20 |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| C22:4 n-6 | 0.05 | 0.18 | 0.29 | 0.15 | 0.12 | 0.25 | 0.29 | 0.10 |
| C22:5 n-3 | 0.20 | 0.18 | 0.10 | 0.18 | 0.18 | 0.43 | 1.63 | 0.15 |
| C22:6 n-3 | 1.46 | 2.19 | 0.24 | 0.38 | 0.32 | 0.25 | 0.40 | 0.27 |
| SFA | 23.77 | 19.05 | 30.93 | 27.45 | 27.68 | 31.30 | 28.97 | 31.02 |
| MUFA | 28.82 | 24.54 | 31.29 | 28.17 | 33.71 | 37.79 | 27.38 | 36.37 |
| PUFA | 47.41 | 56.41 | 37.77 | 44.38 | 38.60 | 30.90 | 43.65 | 32.61 |

[1]According to De Blas *et al.* 2013 (g /kg of diet). Nitrogen-free extracts: DM−( ash + crude protein + crude fat + crude fiber).

FA= fatty acids, SFA = sum of saturated FA, MUFA = sum of monounsaturated FA, PUFA = sum of polyunsaturated FA.

**Supplementary Table 2.** Fatty acids composition of longissimus dorsi muscle (g/100 g total fatty acids).

| | | Groups | | | | | | | | | | *P*-value | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | VLBIW | | LBIW | | MBIW | | HBIW | | | | Contrasts | | | | | | |
| Item | *n* | Females | Males | Females | Males | Females | Males | Females | Males | RMSE | | 1 | 2 | 3 | 4 | 5 | Int |
| **Neutral Lipids** | | | | | | | | | | | | | | | | | | |
| C14:0 | 232 | 1.54 | 1.51 | 1.50 | 1.47 | 1.51 | 1.51 | 1.46 | 1.44 | 0.14 | | ns | ns | ns | ns | 0.02 | ns |
| C16:0 | 232 | 25.51 AB | 24.48 AB | 25.72 AB | 24.28 AB | 25.96 A | 25.71 AB | 25.39 AB | 24.06 B | 2.23 | | 0.01 | ns | ns | ns | 0.008 | 0.01 |
| C16:1 n-9 | 232 | 0.20 | 0.20 | 0.20 | 0.22 | 0.22 | 0.21 | 0.22 | 0.21 | 0.04 | | ns | ns | ns | ns | ns | ns |
| C16:1 n-7 | 232 | 4.40 AB | 4.67 AB | 4.33 B | 4.87 A | 4.51 AB | 4.48 AB | 4.52 AB | 4.87 A | 0.64 | | 0.02 | ns | ns | ns | t | ns |
| C17:0 | 232 | 0.14 | 0.15 | 0.14 | 0.15 | 0.12 | 0.14 | 0.12 | 0.15 | 0.05 | | 0.03 | ns | ns | ns | ns | ns |
| C17:1 | 232 | 0.18 ABC | 0.19 ABC | 0.18 ABC | 0.21 A | 0.15 BC | 0.17 ABC | 0.15 C | 0.20 AB | 0.06 | | 0.01 | ns | ns | ns | ns | ns |
| C18:0 | 232 | 11.25 | 10.81 | 11.23 | 10.34 | 11.02 | 11.07 | 10.98 | 10.41 | 1.08 | | 0.02 | ns | ns | ns | t | ns |
| C18:1 n-9 | 232 | 47.58 | 48.47 | 47.60 | 48.67 | 47.42 | 47.40 | 48.14 | 48.85 | 2.20 | | ns | ns | ns | ns | 0.008 | ns |
| C18:1 n-7 | 232 | 4.66 BC | 4.91 ABC | 4.60 C | 5.12 AB | 4.80 ABC | 4.73 ABC | 4.82 ABC | 5.15 A | 0.58 | | 0.02 | ns | ns | ns | 0.004 | 0.02 |
| C18:2 n-6 | 232 | 2.62 | 2.68 | 2.60 | 2.73 | 2.45 | 2.68 | 2.40 | 2.73 | 0.53 | | t | ns | ns | ns | ns | ns |
| C18:3 n-3 | 232 | 0.44 B | 0.47 AB | 0.46 AB | 0.48 A | 0.44 B | 0.46 AB | 0.44 B | 0.47 AB | 0.04 | | 0.003 | ns | ns | 0.04 | ns | ns |
| C20:0 | 232 | 0.20 | 0.19 | 0.19 | 0.20 | 0.17 | 0.19 | 0.16 | 0.18 | 0.05 | | ns | ns | ns | ns | ns | ns |
| C20:1 n-9 | 232 | 0.97 | 0.97 | 0.92 | 0.96 | 0.93 | 0.94 | 0.90 | 0.94 | 0.11 | | ns | ns | ns | ns | ns | ns |
| C20:3 n-6 | 232 | nd | nd | nd | nd | nd | nd | nd | nd | - | | - | - | - | - | - | - |
| C20:4 n-6 | 232 | 0.15 | 0.15 | 0.16 | 0.13 | 0.13 | 0.15 | 0.14 | 0.15 | 0.06 | | ns | ns | ns | ns | ns | ns |
| C20:5 n-3 | 232 | nd | nd | nd | nd | nd | nd | nd | nd | - | | - | - | - | - | - | - |
| C22:4 n-6 | 232 | nd | nd | nd | nd | nd | nd | nd | nd | - | | - | - | - | - | - | - |
| C22:5 n-3 | 232 | 0.09 | 0.10 | 0.11 | 0.11 | 0.11 | 0.11 | 0.09 | 0.11 | 0.04 | | ns | ns | ns | ns | ns | ns |
| C22:6 n-3 | 232 | 0.06 | 0.07 | 0.07 | 0.06 | 0.06 | 0.07 | 0.07 | 0.07 | 0.05 | | ns | ns | ns | ns | ns | ns |
| SFA | 232 | 38.65 | 37.14 | 38.78 | 36.44 | 38.78 | 38.61 | 38.11 | 36.24 | 3.22 | | 0.01 | ns | ns | ns | 0.01 | 0.02 |
| MUFA | 232 | 57.98 | 59.40 | 57.82 | 60.05 | 58.03 | 57.92 | 58.75 | 60.23 | 3.02 | | 0.03 | ns | ns | ns | 0.007 | 0.02 |
| PUFA | 232 | 3.37 | 3.46 | 3.40 | 3.51 | 3.19 | 3.47 | 3.14 | 3.52 | 0.61 | | t | ns | ns | ns | ns | ns |

| | | | | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Σn3 | 232 | 0.59 | | 0.64 | | 0.65 | | 0.65 | | 0.61 | | 0.64 | | 0.60 | | 0.65 | | 0.09 | t | ns | ns | ns | ns | ns |
| Σn6 | 232 | 2.78 | | 2.82 | | 2.75 | | 2.86 | | 2.58 | | 2.83 | | 2.54 | | 2.88 | | 0.56 | t | ns | ns | ns | ns | ns |
| Σn6/Σn3 | 232 | 4.68 | | 4.52 | | 4.33 | | 4.38 | | 4.27 | | 4.46 | | 4.28 | | 4.48 | | 0.82 | ns | ns | ns | ns | ns | ns |
| IU | 232 | 65.98 | | 67.66 | | 66.01 | | 68.41 | | 65.69 | | 66.22 | | 66.32 | | 68.64 | | 3.64 | 0.01 | ns | ns | ns | 0.02 | 0.03 |
| MUFA/SFA | 232 | 1.51 | AB | 1.64 | AB | 1.50 | B | 1.71 | A | 1.51 | AB | 1.51 | AB | 1.55 | AB | 1.70 | A | 0.25 | 0.006 | ns | ns | ns | 0.01 | 0.008 |
| C18:1/C18:0 | 232 | 4.72 | B | 5.05 | AB | 4.67 | B | 5.36 | A | 4.79 | AB | 4.76 | AB | 4.86 | AB | 5.30 | AB | 0.76 | 0.02 | ns | ns | ns | 0.03 | 0.02 |

| | | Groups | | | | | | | | | *P*-value | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | VLBIW | | LBIW | | MBIW | | HBIW | | | **Contrasts** | | | | | |
| Item | *n* | Females | Males | Females | Males | Females | Males | Females | Males | RMSE | **1** | **2** | **3** | **4** | **5** | **Int** |
| **Polar Lipids** | | | | | | | | | | | | | | | | |
| C14:0 | 232 | 2.09 B | 2.89 A | 2.84 A | 2.82 A | 2.80 A | 2.62 A | 2.68 A | 2.63 A | 0.67 | ns | ns | ns | ns | ns | ns |
| C16:0 | 232 | 19.62 A | 18.52 AB | 18.76 AB | 17.94 B | 18.62 AB | 18.53 AB | 18.72 AB | 18.21 B | 1.50 | 0.02 | ns | ns | ns | ns | ns |
| C16:1 n-9 | 232 | 0.32 | 0.31 | 0.30 | 0.40 | 0.31 | 0.30 | 0.31 | 0.34 | 0.14 | ns | ns | ns | ns | ns | ns |
| C16:1 n-7 | 232 | 1.24 AB | 1.34 A | 1.10 BC | 1.14 ABC | 1.04 BC | 1.16 ABC | 0.98 C | 1.14 ABC | 0.29 | t | 0.01 | t | ns | ns | ns |
| C17:0 | 232 | 0.50 | 0.58 | 0.52 | 0.58 | 0.49 | 0.54 | 0.52 | 0.53 | 0.12 | 0.02 | ns | ns | ns | ns | ns |
| C17:1 | 232 | 0.84 | 0.70 | 0.86 | 0.71 | 0.86 | 1.14 | 1.00 | 0.73 | 0.87 | ns | ns | ns | ns | ns | ns |
| C18:0 | 232 | 9.59 A | 9.20 AB | 8.88 B | 8.93 B | 8.92 B | 9.11 AB | 8.86 B | 8.78 B | 0.75 | ns | 0.02 | 0.04 | ns | ns | ns |
| C18:1 n-9 | 232 | 16.90 A | 16.80 A | 15.29 AB | 16.43 A | 14.51 B | 15.36 AB | 14.62 B | 16.08 AB | 2.21 | 0.04 | 0.01 | ns | ns | ns | 0.003 |
| C18:1 n-7 | 232 | 3.48 | 3.65 | 3.52 | 3.51 | 3.44 | 3.45 | 3.47 | 3.50 | 0.36 | ns | ns | ns | ns | ns | ns |
| C18:2 n-6 | 232 | 27.86 B | 28.43 AB | 29.46 AB | 28.96 AB | 29.92 A | 29.44 AB | 30.16 A | 29.71 A | 2.22 | ns | 0.02 | ns | ns | ns | ns |
| C18:3 n-3 | 232 | 0.54 | 0.57 | 0.54 | 0.56 | 0.54 | 0.55 | 0.54 | 0.54 | 0.09 | ns | ns | ns | ns | ns | ns |
| C20:0 | 232 | 0.22 | 0.22 | 0.23 | 0.24 | 0.21 | 0.26 | 0.22 | 0.23 | 0.06 | ns | ns | ns | ns | ns | ns |
| C20:1 n-9 | 232 | 0.38 A | 0.38 AB | 0.32 AB | 0.36 AB | 0.30 B | 0.34 AB | 0.33 AB | 0.34 AB | 0.10 | ns | t | ns | ns | ns | ns |
| C20:3 n-6 | 232 | 1.28 | 1.33 | 1.32 | 1.32 | 1.38 | 1.29 | 1.26 | 1.37 | 0.18 | ns | ns | ns | ns | ns | ns |
| C20:4 n-6 | 232 | 11.17 | 10.94 | 11.70 | 11.58 | 12.13 | 11.50 | 11.66 | 11.42 | 1.92 | ns | ns | ns | ns | ns | ns |
| C20:5 n-3 | 232 | 0.47 | 0.50 | 0.47 | 0.51 | 0.52 | 0.49 | 0.49 | 0.49 | 0.08 | ns | ns | ns | ns | ns | ns |

| | | | | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| C22:4 n-6 | 232 | 1.26 | B | 1.26 | B | 1.39 | AB | 1.43 | AB | 1.47 | A | 1.36 | AB | 1.46 | A | 1.42 | AB | 0.23 | ns | 0.009 | 0.04 | ns | ns | ns |
| C22:5 n-3 | 232 | 1.36 | B | 1.51 | AB | 1.59 | AB | 1.64 | A | 1.63 | AB | 1.60 | AB | 1.73 | A | 1.63 | AB | 0.33 | ns | 0.02 | t | ns | ns | ns |
| C22:6 n-3 | 232 | 0.87 | | 0.88 | | 0.90 | | 0.95 | | 0.92 | | 0.95 | | 0.97 | | 0.92 | | 0.18 | ns | ns | ns | ns | ns | ns |
| SFA | 232 | 32.02 | A | 31.40 | AB | 31.23 | AB | 30.51 | B | 31.04 | AB | 31.06 | AB | 31.00 | AB | 30.38 | B | 1.55 | t | 0.04 | t | ns | ns | ns |
| MUFA | 232 | 23.16 | A | 23.18 | A | 21.40 | AB | 22.54 | AB | 20.47 | B | 21.75 | AB | 20.72 | B | 22.12 | AB | 2.72 | t | 0.02 | ns | ns | ns | 0.04 |
| PUFA | 232 | 44.81 | B | 45.41 | B | 47.37 | AB | 46.95 | AB | 48.49 | A | 47.20 | AB | 48.28 | A | 47.50 | AB | 3.41 | ns | 0.007 | t | ns | ns | ns |
| Σn3 | 232 | 3.25 | B | 3.44 | AB | 3.50 | AB | 3.65 | AB | 3.60 | AB | 3.60 | AB | 3.72 | A | 3.57 | AB | 0.47 | ns | 0.04 | ns | ns | ns | ns |
| Σn6 | 232 | 41.57 | B | 41.97 | B | 43.87 | AB | 43.30 | AB | 44.90 | A | 43.60 | AB | 44.55 | A | 43.93 | AB | 3.13 | ns | 0.008 | t | ns | ns | ns |
| Σn6/Σn3 | 232 | 12.88 | | 12.24 | | 12.71 | | 11.97 | | 12.55 | | 12.29 | | 12.19 | | 12.43 | | 1.30 | ns | ns | ns | ns | ns | ns |
| IU | 232 | 1.48 | B | 1.50 | AB | 1.54 | AB | 1.55 | AB | 1.57 | A | 1.54 | AB | 1.56 | AB | 1.55 | AB | 0.09 | ns | 0.02 | t | ns | ns | ns |
| MUFA/SFA | 232 | 0.72 | AB | 0.74 | A | 0.69 | AB | 0.74 | A | 0.66 | B | 0.70 | AB | 0.67 | AB | 0.73 | AB | 0.09 | 0.009 | ns | ns | ns | ns | 0.001 |
| C18:1/C18:0 | 232 | 2.12 | | 2.23 | | 2.14 | | 2.24 | | 2.02 | | 2.09 | | 2.05 | | 2.26 | | 0.33 | 0.04 | ns | ns | ns | t | 0.02 |

VLBIW= Very low birth-Wt, LBIW= Low birth-Wt, MBIW= Medium birth-Wt, HBIW= High birth-Wt. Wt=Weight. Nd= Not detectable

SFA = sum of saturated fatty acids, MUFA = sum of monounsaturated fatty acids, PUFA = sum of polyunsaturated fatty acids,

UI = unsaturation index = 1 × (% monoenoics) + 2 × (% dienoics) + 3 × (% trienoics) + 4 × (% tetraenoics) + 5 × (% pentaenoics) + 6 × (% hexaenoics).

RMSE = root-mean-square error. Ns= not significant, t= 0.1> *P*>0.05. Different letters in a line indicate significant differences (*P*<0.05).

Contrast 1: Females-Males; C2: VLBIW-(LBIW+MBIW+HBIW); C3: VLBIW-LBIW; C4: LBIW-(MBIW+HBIW); C5: MBIW-HBIW; Int: Interaction birth-Wt and sex.

**Supplementary Table 3.** Fatty acids composition of subcutaneous fat (g/100 g total fatty acids).

| | | Groups | | | | | | | | | | | | | | *P*-value Contrasts | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | VLBIW | | LBIW | | MBIW | | HBIW | | | | | | | | | | | | | |
| Item | *n* | Females | | Males | | Females | | Males | | Females | | Males | | Females | | Males | | RMSE | 1 | 2 | 3 | 4 | 5 | Int |
| Outer layer | | | | | | | | | | | | | | | | | | | | | | | | |
| C14:0 | 232 | 1.58 | A | 1.54 | AB | 1.54 | AB | 1.50 | AB | 1.55 | AB | 1.51 | AB | 1.51 | AB | 1.49 | B | 0.11 | t | ns | ns | ns | ns | ns |
| C16:0 | 232 | 24.84 | AB | 24.10 | ABC | 24.80 | AB | 23.82 | C | 24.95 | A | 24.50 | ABC | 24.77 | AB | 23.87 | BC | 1.18 | 0.001 | ns | ns | ns | t | 0.003 |
| C16:1 n-9 | 232 | 0.30 | | 0.36 | | 0.31 | | 0.41 | | 0.38 | | 0.34 | | 0.33 | | 0.36 | | 0.16 | ns | ns | ns | ns | ns | ns |
| C16:1 n-7 | 232 | 2.73 | B | 2.78 | B | 2.74 | B | 3.11 | AB | 2.82 | AB | 2.81 | AB | 2.73 | B | 3.02 | AB | 0.40 | 0.02 | ns | ns | ns | ns | ns |
| C17:0 | 232 | 0.27 | C | 0.31 | A | 0.27 | BC | 0.30 | ABC | 0.28 | ABC | 0.28 | ABC | 0.28 | ABC | 0.31 | AB | 0.05 | 0.003 | ns | ns | ns | ns | ns |
| C17:1 | 232 | 0.31 | C | 0.34 | ABC | 0.30 | C | 0.35 | AB | 0.32 | BC | 0.33 | ABC | 0.31 | C | 0.36 | A | 0.05 | 0.0001 | ns | ns | ns | ns | ns |
| C18:0 | 232 | 11.30 | AB | 11.46 | AB | 11.64 | A | 10.66 | B | 11.57 | A | 11.21 | AB | 11.64 | AB | 10.78 | AB | 1.08 | 0.01 | ns | ns | ns | ns | 0.02 |
| C18:1 n-9 | 232 | 44.66 | AB | 45.32 | A | 44.53 | AB | 45.45 | A | 44.13 | B | 45.04 | AB | 44.50 | AB | 45.61 | A | 1.37 | 0.0004 | ns | ns | ns | t | 0.0004 |
| C18:1 n-7 | 232 | 3.46 | | 3.34 | | 3.27 | | 3.81 | | 3.40 | | 3.29 | | 3.04 | | 3.40 | | 0.95 | ns | ns | ns | ns | ns | ns |
| C18:2 n-6 | 232 | 8.49 | AB | 8.23 | B | 8.52 | AB | 8.44 | AB | 8.55 | AB | 8.54 | AB | 8.82 | A | 8.71 | AB | 0.66 | ns | ns | ns | ns | t | ns |
| C18:3 n-3 | 232 | 0.63 | B | 0.63 | B | 0.64 | AB | 0.65 | AB | 0.65 | AB | 0.65 | AB | 0.67 | A | 0.67 | A | 0.05 | ns | t | ns | t | 0.01 | ns |
| C20:0 | 232 | 0.20 | B | 0.22 | A | 0.19 | B | 0.19 | B | 0.19 | B | 0.20 | B | 0.19 | B | 0.19 | B | 0.03 | ns | 0.02 | t | ns | ns | ns |
| C20:1 n-9 | 232 | 1.09 | BC | 1.22 | A | 1.09 | BC | 1.15 | AB | 1.04 | C | 1.15 | AB | 1.05 | C | 1.07 | BC | 0.12 | 0.0003 | t | ns | t | ns | ns |
| C20:4 n-6 | 232 | 0.15 | | 0.16 | | 0.16 | | 0.16 | | 0.17 | | 0.16 | | 0.16 | | 0.16 | | 0.03 | ns | ns | ns | ns | ns | ns |
| SFA | 232 | 38.19 | AB | 37.63 | ABC | 38.43 | A | 36.47 | C | 38.55 | A | 37.70 | ABC | 38.39 | A | 36.63 | BC | 2.11 | 0.001 | ns | ns | ns | ns | 0.003 |
| MUFA | 232 | 52.55 | BC | 53.35 | AB | 52.25 | C | 54.28 | A | 52.08 | C | 52.96 | ABC | 51.95 | C | 53.82 | AB | 1.89 | <.0001 | ns | ns | ns | ns | 0.0003 |
| PUFA | 232 | 9.27 | AB | 9.03 | B | 9.32 | AB | 9.24 | AB | 9.37 | AB | 9.35 | AB | 9.66 | A | 9.54 | AB | 0.72 | ns | ns | ns | ns | t | ns |
| Σn3 | 232 | 0.63 | B | 0.63 | B | 0.64 | AB | 0.65 | AB | 0.65 | AB | 0.65 | AB | 0.67 | A | 0.67 | A | 0.05 | ns | t | ns | t | 0.01 | ns |
| Σn6 | 232 | 8.63 | AB | 8.39 | B | 8.68 | AB | 8.60 | AB | 8.72 | AB | 8.69 | AB | 8.99 | A | 8.87 | AB | 0.68 | ns | ns | ns | ns | t | ns |
| Σn6/Σn3 | 232 | 13.69 | A | 13.30 | B | 13.56 | AB | 13.28 | B | 13.44 | AB | 13.34 | AB | 13.33 | AB | 13.23 | B | 0.46 | 0.009 | ns | ns | ns | ns | ns |
| IU | 232 | 0.72 | | 0.72 | | 0.72 | | 0.74 | | 0.72 | | 0.73 | | 0.72 | | 0.74 | | 0.03 | 0.02 | ns | ns | ns | t | 0.02 |
| MUFA/SFA | 232 | 1.38 | BC | 1.43 | ABC | 1.36 | C | 1.50 | A | 1.36 | C | 1.41 | ABC | 1.36 | C | 1.48 | AB | 0.14 | 0.0003 | ns | ns | ns | ns | 0.001 |

| | | | | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| C18:1/C18:0 | 232 | 4.29 | ABC | 4.36 | ABC | 4.13 | C | 4.71 | A | 4.15 | BC | 4.35 | ABC | 4.15 | BC | 4.61 | AB | 0.58 | 0.002 | ns | ns | ns | ns | 0.006 |

| | | Groups | | | | | | | | | | | | | | *P*-value | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | VLBIW | | | | LBIW | | | | MBIW | | | | HBIW | | | | | Contrasts | | | | | |
| Item | *n* | Females | | Males | | Females | | Males | | Females | | Males | | Females | | Males | | RMSE | 1 | 2 | 3 | 4 | 5 | Int |
| Inner layer | | | | | | | | | | | | | | | | | | | | | | | | |
| C14:0 | 232 | 1.54 | | 1.46 | | 1.53 | | 1.46 | | 1.51 | | 1.48 | | 1.48 | | 1.44 | | 0.14 | 0.03 | ns | ns | ns | ns | ns |
| C16:0 | 232 | 25.97 | A | 24.49 | C | 25.98 | A | 24.86 | BC | 25.96 | A | 25.60 | AB | 25.97 | A | 24.87 | BC | 1.28 | <.0001 | ns | ns | ns | ns | 0.0003 |
| C16:1 n-9 | 232 | 0.24 | | 0.26 | | 0.26 | | 0.26 | | 0.26 | | 0.26 | | 0.25 | | 0.27 | | 0.05 | ns | ns | ns | ns | ns | ns |
| C16:1 n-7 | 232 | 2.43 | AB | 2.56 | AB | 2.37 | B | 2.66 | AB | 2.39 | B | 2.54 | AB | 2.46 | AB | 2.73 | A | 0.40 | 0.005 | ns | ns | ns | t | ns |
| C17:0 | 232 | 0.23 | D | 0.28 | A | 0.23 | D | 0.27 | AB | 0.24 | BCD | 0.25 | BCD | 0.23 | CD | 0.26 | ABC | 0.04 | <.0001 | ns | ns | ns | ns | ns |
| C17:1 | 232 | 0.24 | B | 0.29 | A | 0.24 | B | 0.29 | A | 0.25 | B | 0.26 | AB | 0.24 | B | 0.29 | A | 0.04 | <.0001 | ns | ns | ns | ns | ns |
| C18:0 | 232 | 13.29 | AB | 12.67 | AB | 13.52 | A | 12.32 | B | 13.62 | A | 12.71 | AB | 13.53 | A | 12.29 | B | 1.28 | <.0001 | ns | ns | ns | ns | <.0001 |
| C18:1 n-9 | 232 | 44.03 | B | 45.54 | A | 44.00 | | 45.32 | A | 43.68 | B | 44.61 | AB | 43.76 | B | 45.32 | A | 1.62 | <.0001 | ns | ns | ns | ns | <.0001 |
| C18:1 n-7 | 232 | 2.65 | AB | 2.71 | AB | 2.57 | B | 2.79 | AB | 2.58 | AB | 2.77 | AB | 2.55 | B | 2.89 | A | 0.38 | 0.004 | ns | ns | ns | ns | 0.01 |
| C18:2 n-6 | 232 | 6.99 | | 7.25 | | 6.92 | | 7.33 | | 7.18 | | 7.13 | | 7.22 | | 7.31 | | 0.54 | t | ns | ns | ns | ns | ns |
| C18:3 n-3 | 232 | 0.81 | C | 0.85 | ABC | 0.82 | BC | 0.88 | A | 0.84 | ABC | 0.84 | ABC | 0.85 | AB | 0.86 | A | 0.05 | 0.002 | ns | ns | ns | ns | ns |
| C20:0 | 232 | 0.21 | | 0.23 | | 0.21 | | 0.22 | | 0.21 | | 0.21 | | 0.21 | | 0.21 | | 0.03 | t | ns | ns | ns | ns | ns |
| C20:1 n-9 | 232 | 1.26 | A | 1.26 | A | 1.22 | AB | 1.20 | AB | 1.14 | AB | 1.20 | AB | 1.12 | B | 1.12 | B | 0.15 | ns | 0.03 | ns | t | t | ns |
| C20:4 n-6 | 232 | 0.12 | B | 0.13 | AB | 0.14 | A | 0.14 | A | 0.13 | AB | 0.13 | AB | 0.14 | A | 0.14 | A | 0.02 | ns | 0.04 | t | ns | t | ns |
| SFA | 232 | 41.24 | A | 39.13 | B | 41.48 | A | 39.14 | B | 41.54 | A | 40.25 | AB | 41.42 | A | 39.08 | B | 2.32 | <.0001 | ns | ns | ns | ns | <.0001 |
| MUFA | 232 | 50.84 | B | 52.63 | A | 50.65 | B | 52.51 | A | 50.30 | B | 51.64 | AB | 50.37 | B | 52.61 | A | 2.04 | <.0001 | ns | ns | ns | ns | <.0001 |
| PUFA | 232 | 7.92 | | 8.24 | | 7.87 | | 8.35 | | 8.16 | | 8.11 | | 8.20 | | 8.31 | | 0.59 | t | ns | ns | ns | ns | ns |
| Σn3 | 232 | 0.81 | C | 0.85 | AB | 0.82 | CB | 0.88 | A | 0.84 | ABC | 0.84 | ABC | 0.85 | ABC | 0.86 | A | 0.05 | 0.002 | ns | ns | ns | ns | 0.02 |
| Σn6 | 232 | 7.11 | | 7.39 | | 7.05 | | 7.47 | | 7.32 | | 7.27 | | 7.36 | | 7.45 | | 0.55 | t | ns | ns | ns | ns | ns |
| Σn6/Σn3 | 232 | 8.71 | | 8.64 | | 8.60 | | 8.49 | | 8.67 | | 8.63 | | 8.66 | | 8.63 | | 0.31 | ns | ns | ns | ns | ns | ns |
| IU | 232 | 0.68 | B | 0.70 | A | 0.67 | B | 0.70 | A | 0.68 | B | 0.69 | AB | 0.68 | B | 0.70 | A | 0.03 | <.0001 | ns | ns | ns | ns | 0.0001 |

| | | | | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| MUFA/SFA | 232 | 1.23 | B | 1.36 | A | 1.22 | B | 1.36 | A | 1.21 | B | 1.29 | AB | 1.22 | B | 1.36 | A | 0.13 | <.0001 | ns | ns | ns | t | <.0001 |
| C18:1/C18:0 | 232 | 3.55 | BC | 3.91 | AB | 3.47 | C | 3.98 | A | 3.42 | C | 3.77 | ABC | 3.45 | C | 4.01 | A | 0.51 | <.0001 | ns | ns | ns | ns | <.0001 |

VLBIW= Very low birth-Wt, LBIW= Low birth-Wt, MBIW= Medium birth-Wt, HBIW= High birth-Wt. Wt=Weight.

SFA = sum of saturated fatty acids, MUFA = sum of monounsaturated fatty acids, PUFA = sum of polyunsaturated fatty acids,

UI = unsaturation index = 1 × (% monoenoics) + 2 × (% dienoics) + 3 × (% trienoics) + 4 × (% tetraenoics) + 5 × (% pentaenoics) + 6 × (% hexaenoics).

RMSE = root-mean-square error. Ns= not significant, t= 0.1> *P*>0.05. Different letters in a line indicate significant differences (*P*<0.05).

Contrast 1: Females-Males; C2: VLBIW-(LBIW+MBIW+HBIW); C3: VLBIW-LBIW; C4: LBIW-(MBIW+HBIW); C5: MBIW-HBIW; Int: Interaction birth-Wt and sex.

**Supplementary Table 4.** Fatty acids composition of liver (g/100 g total fatty acids).

| | | Groups | | | | | | | | | | *P*-value Contrasts | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | VLBIW | | LBIW | | MBIW | | HBIW | | | | | | | | | |
| Item | *n* | Females | Males | Females | Males | Females | Males | Females | Males | RMSE | 1 | 2 | 3 | 4 | 5 | Int |
| Neutral Lipids | | | | | | | | | | | | | | | | |
| C14:0 | 232 | 0.62 $^{B}$ | 0.80 $^{AB}$ | 0.86 $^{AB}$ | 0.94 $^{A}$ | 0.80 $^{AB}$ | 0.68 $^{AB}$ | 0.68 $^{AB}$ | 0.82 $^{AB}$ | 0.27 | ns | ns | ns | 0.02 | ns | ns |
| C16:0 | 232 | 19.21 | 20.71 | 20.40 | 21.54 | 20.78 | 19.49 | 19.58 | 20.80 | 2.46 | ns | ns | ns | ns | ns | ns |
| C16:1 n-9 | 232 | 0.52 $^{B}$ | 0.63 $^{AB}$ | 0.72 $^{AB}$ | 0.79 $^{A}$ | 0.68 $^{AB}$ | 0.58 $^{AB}$ | 0.61 $^{AB}$ | 0.73 $^{AB}$ | 0.21 | ns | ns | t | 0.04 | ns | ns |
| C16:1 n-7 | 232 | 1.33 $^{B}$ | 1.73 $^{AB}$ | 1.69 $^{AB}$ | 2.13 $^{A}$ | 1.62 $^{AB}$ | 1.51 $^{B}$ | 1.42 $^{B}$ | 1.81 $^{AB}$ | 0.52 | 0.04 | ns | ns | 0.01 | ns | ns |
| C17:0 | 232 | 0.54 | 0.58 | 0.57 | 0.52 | 0.60 | 0.62 | 0.70 | 0.59 | 0.16 | ns | ns | ns | 0.03 | ns | ns |
| C17:1 | 232 | 0.36 $^{AB}$ | 0.37 $^{A}$ | 0.32 $^{AB}$ | 0.38 $^{A}$ | 0.31 $^{AB}$ | 0.33 $^{AB}$ | 0.30 $^{B}$ | 0.35 $^{AB}$ | 0.06 | 0.02 | ns | ns | t | ns | ns |
| C18:0 | 232 | 25.77 $^{A}$ | 23.16 $^{AB}$ | 20.91 $^{B}$ | 19.42 $^{B}$ | 23.08 $^{AB}$ | 23.32 $^{AB}$ | 23.64 $^{AB}$ | 22.07 $^{AB}$ | 3.96 | ns | ns | 0.02 | 0.003 | ns | ns |
| C18:1 n-9 | 232 | 24.34 $^{B}$ | 28.65 $^{AB}$ | 29.11 $^{AB}$ | 31.50 $^{A}$ | 26.85 $^{AB}$ | 25.95 $^{AB}$ | 24.85 $^{B}$ | 28.24 $^{AB}$ | 5.50 | ns | ns | ns | 0.005 | ns | ns |
| C18:1 n-7 | 232 | 1.59 | 1.76 | 1.53 | 1.73 | 1.67 | 1.69 | 1.66 | 1.88 | 0.31 | t | ns | ns | ns | ns | ns |
| C18:2 n-6 | 232 | 10.94 | 9.76 | 11.21 | 10.25 | 10.60 | 11.04 | 11.77 | 10.37 | 1.87 | ns | ns | ns | ns | ns | ns |
| C18:3 n-3 | 232 | 0.21 | 0.24 | 0.31 | 0.30 | 0.26 | 0.28 | 0.27 | 0.26 | 0.09 | ns | ns | 0.04 | t | ns | ns |
| C20:0 | 232 | 0.35 | 0.33 | 0.25 | 0.38 | 0.39 | 0.29 | 0.28 | 0.33 | 0.26 | ns | ns | ns | ns | ns | ns |
| C20:1 n-9 | 232 | 0.72 | 0.71 | 0.54 | 0.80 | 0.67 | 0.58 | 0.60 | 0.70 | 0.32 | ns | ns | ns | ns | ns | ns |
| C20:3 n-6 | 232 | 0.67 $^{AB}$ | 0.47 $^{AB}$ | 0.61 $^{AB}$ | 0.38 $^{B}$ | 0.52 $^{AB}$ | 0.53 $^{AB}$ | 0.76 $^{A}$ | 0.51 $^{AB}$ | 0.27 | 0.01 | ns | ns | ns | 0.04 | ns |
| C20:4 n-6 | 232 | 9.43 | 7.62 | 8.43 | 6.81 | 8.58 | 10.06 | 9.84 | 8.03 | 3.40 | ns | ns | ns | t | ns | ns |
| C20:5 n-3 | 232 | 0.24 | 0.20 | 0.20 | 0.17 | 0.23 | 0.24 | 0.25 | 0.23 | 0.07 | ns | ns | ns | 0.007 | ns | ns |
| C22:4 n-6 | 232 | 0.66 | 0.56 | 0.65 | 0.55 | 0.63 | 0.77 | 0.73 | 0.60 | 0.23 | ns | ns | ns | ns | ns | ns |
| C22:5 n-3 | 232 | 0.90 | 0.77 | 0.89 | 0.65 | 0.82 | 1.06 | 1.10 | 0.76 | 0.51 | ns | ns | ns | ns | ns | ns |
| C22:6 n-3 | 232 | 0.94 | 0.95 | 0.80 | 0.76 | 0.89 | 1.00 | 0.96 | 0.92 | 0.32 | ns | ns | ns | 0.04 | ns | ns |
| SFA | 232 | 46.69 $^{A}$ | 45.58 $^{AB}$ | 42.99 $^{AB}$ | 42.81 $^{B}$ | 45.66 $^{AB}$ | 44.39 $^{AB}$ | 44.88 $^{AB}$ | 44.61 $^{AB}$ | 3.25 | ns | ns | 0.03 | 0.02 | ns | ns |
| MUFA | 232 | 28.87 $^{B}$ | 33.85 $^{AB}$ | 33.91 $^{AB}$ | 37.33 $^{A}$ | 31.80 $^{AB}$ | 30.64 $^{AB}$ | 29.43 $^{B}$ | 33.72 $^{AB}$ | 6.37 | t | ns | ns | 0.01 | ns | ns |
| PUFA | 232 | 23.99 | 20.57 | 23.10 | 19.87 | 22.54 | 24.97 | 25.69 | 21.67 | 6.26 | ns | ns | ns | ns | ns | ns |

| | | | | | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Σn3 | 232 | 2.29 | | 2.15 | | 2.20 | | 1.88 | | 2.21 | | 2.58 | | 2.57 | | 2.16 | | 0.82 | ns | ns | ns | t | ns | ns |
| Σn6 | 232 | 21.70 | | 18.41 | | 20.90 | | 17.99 | | 20.34 | | 22.39 | | 23.11 | | 19.50 | | 5.47 | ns | ns | ns | ns | ns | ns |
| Σn6/Σn3 | 232 | 9.68 | | 8.62 | | 9.76 | | 9.95 | | 9.53 | | 8.97 | | 9.36 | | 9.46 | | 1.25 | ns | ns | ns | t | ns | ns |
| IU | 232 | 1.05 | | 0.99 | | 1.06 | | 0.98 | | 1.03 | | 1.11 | | 1.11 | | 1.02 | | 0.17 | ns | ns | ns | ns | ns | ns |
| MUFA/SFA | 232 | 0.62 | B | 0.75 | AB | 0.81 | AB | 0.88 | A | 0.70 | AB | 0.70 | AB | 0.66 | B | 0.77 | AB | 0.17 | t | ns | 0.04 | 0.0009 | ns | ns |
| C18:1/C18:0 | 232 | 1.07 | C | 1.35 | ABC | 1.70 | AB | 1.87 | A | 1.30 | ABC | 1.24 | BC | 1.17 | BC | 1.46 | ABC | 0.54 | ns | ns | 0.02 | 0.0003 | ns | ns |

| | *n* | Groups | | | | | | | | | *P*-value | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | VLBIW | | LBIW | | MBIW | | HBIW | | | Contrasts | | | | | | |
| Item | *n* | Females | Males | Females | Males | Females | Males | Females | Males | RMSE | 1 | 2 | 3 | 4 | 5 | Int |
| Polar Lipids | | | | | | | | | | | | | | | | |
| C14:0 | 232 | 0.22 B | 0.34 A | 0.27 AB | 0.29 AB | 0.29 AB | 0.28 AB | 0.28 AB | 0.33 A | 0.07 | 0.02 | ns | ns | ns | ns | ns |
| C16:0 | 232 | 15.94 C | 18.57 AB | 17.41 ABC | 19.94 A | 18.20 ABC | 17.25 BC | 17.79 ABC | 18.90 AB | 2.25 | 0.02 | ns | ns | ns | ns | ns |
| C16:1 n-9 | 232 | 0.20 B | 0.26 AB | 0.24 AB | 0.25 AB | 0.25 AB | 0.23 AB | 0.24 AB | 0.26 A | 0.05 | ns | ns | ns | ns | ns | ns |
| C16:1 n-7 | 232 | 0.57 B | 0.76 A | 0.61 B | 0.79 A | 0.69 AB | 0.65 AB | 0.65 AB | 0.76 A | 0.14 | 0.001 | ns | ns | ns | ns | ns |
| C17:0 | 232 | 0.59 | 0.68 | 0.67 | 0.64 | 0.68 | 0.73 | 0.78 | 0.69 | 0.17 | ns | ns | ns | ns | ns | ns |
| C17:1 | 232 | 0.16 C | 0.32 A | 0.22 BC | 0.29 AB | 0.25 ABC | 0.22 BC | 0.21 BC | 0.25 ABC | 0.09 | 0.005 | ns | ns | ns | ns | ns |
| C18:0 | 232 | 32.29 A | 29.72 B | 30.16 B | 28.91 B | 30.22 B | 29.65 B | 29.79 B | 29.69 B | 1.40 | 0.002 | 0.03 | 0.03 | ns | ns | 0.01 |
| C18:1 n-9 | 232 | 15.30 B | 17.88 A | 15.77 AB | 17.28 AB | 16.54 AB | 15.93 AB | 15.15 B | 17.16 AB | 2.24 | 0.02 | ns | ns | ns | ns | ns |
| C18:1 n-7 | 232 | 1.27 D | 1.64 A | 1.34 BCD | 1.59 ABC | 1.42 ABCD | 1.45 ABCD | 1.32 CD | 1.60 AB | 0.24 | 0.0003 | ns | ns | ns | ns | ns |
| C18:2 n-6 | 232 | 13.07 A | 11.49 B | 12.45 AB | 11.82 AB | 11.88 AB | 11.89 AB | 12.86 A | 11.55 B | 1.13 | 0.003 | ns | ns | ns | ns | 0.01 |
| C18:3 n-3 | 232 | 0.15 C | 0.25 A | 0.20 AB | 0.23 AB | 0.20 BC | 0.22 AB | 0.19 BC | 0.21 AB | 0.04 | 0.0001 | ns | ns | ns | ns | ns |
| C20:0 | 232 | 0.28 | 0.33 | 0.34 | 0.32 | 0.32 | 0.35 | 0.33 | 0.36 | 0.10 | ns | ns | ns | ns | ns | ns |
| C20:1 n-9 | 232 | 0.22 B | 0.31 A | 0.25 AB | 0.27 AB | 0.26 AB | 0.24 AB | 0.24 AB | 0.28 AB | 0.07 | t | ns | ns | ns | ns | ns |
| C20:3 n-6 | 232 | 0.86 AB | 0.67 AB | 0.85 AB | 0.56 B | 0.67 AB | 0.65 AB | 0.95 A | 0.70 AB | 0.27 | 0.007 | ns | ns | ns | 0.003 | ns |
| C20:4 n-6 | 232 | 14.58 | 12.78 | 14.80 | 12.95 | 14.00 | 15.43 | 14.85 | 13.19 | 2.98 | ns | ns | ns | ns | ns | ns |
| C20:5 n-3 | 232 | 0.44 | 0.36 | 0.39 | 0.35 | 0.38 | 0.41 | 0.38 | 0.41 | 0.08 | ns | ns | ns | ns | ns | ns |

| | | | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| C22:4 n-6 | 232 | 0.78 | | 0.80 | | 0.89 | | 0.84 | | 0.85 | | 0.97 | | 0.93 | | 0.84 | | 0.18 | ns | ns | ns | ns | ns | ns |
| C22:5 n-3 | 232 | 1.46 | | 1.37 | | 1.65 | | 1.41 | | 1.52 | | 1.77 | | 1.84 | | 1.46 | | 0.56 | ns | ns | ns | ns | ns | ns |
| C22:6 n-3 | 232 | 1.63 | | 1.47 | | 1.50 | | 1.29 | | 1.38 | | 1.68 | | 1.22 | | 1.37 | | 0.41 | ns | ns | ns | ns | 0.005 | ns |
| SFA | 232 | 49.32 | | 49.64 | | 48.84 | | 50.10 | | 49.72 | | 48.25 | | 48.97 | | 49.97 | | 2.43 | ns | ns | ns | ns | ns | ns |
| MUFA | 232 | 17.71 | B | 21.18 | A | 18.43 | AB | 20.46 | AB | 19.42 | AB | 18.73 | AB | 17.81 | B | 20.31 | AB | 2.62 | 0.007 | ns | ns | ns | ns | 0.04 |
| PUFA | 232 | 32.97 | | 29.18 | | 32.73 | | 29.45 | | 30.87 | | 33.02 | | 33.22 | | 29.73 | | 4.53 | t | ns | ns | ns | ns | ns |
| Σn3 | 232 | 3.68 | | 3.44 | | 3.75 | | 3.28 | | 3.48 | | 4.07 | | 3.63 | | 3.45 | | 0.80 | ns | ns | ns | ns | ns | ns |
| Σn6 | 232 | 29.29 | | 25.74 | | 28.98 | | 26.16 | | 27.39 | | 28.94 | | 29.59 | | 26.27 | | 3.88 | 0.04 | ns | ns | ns | ns | ns |
| Σn6/Σn3 | 232 | 7.99 | | 7.60 | | 7.83 | | 8.06 | | 8.10 | | 7.24 | | 8.39 | | 7.82 | | 1.14 | ns | ns | ns | ns | t | 0.01 |
| IU | 232 | 1.28 | | 1.19 | | 1.28 | | 1.18 | | 1.23 | | 1.32 | | 1.28 | | 1.20 | | 0.15 | ns | ns | ns | ns | ns | ns |
| MUFA/SFA | 232 | 0.36 | B | 0.43 | A | 0.38 | AB | 0.41 | AB | 0.39 | AB | 0.39 | AB | 0.36 | B | 0.41 | AB | 0.04 | 0.02 | ns | ns | ns | ns | 0.01 |
| C18:1/C18:0 | 232 | 0.51 | C | 0.66 | A | 0.57 | ABC | 0.66 | A | 0.60 | ABC | 0.59 | ABC | 0.55 | BC | 0.63 | AB | 0.08 | 0.001 | ns | ns | ns | ns | 0.006 |

VLBIW= Very low birth-Wt, LBIW= Low birth-Wt, MBIW= Medium birth-Wt, HBIW= High birth-Wt. Wt=Weight.

SFA = sum of saturated fatty acids, MUFA = sum of monounsaturated fatty acids, PUFA = sum of polyunsaturated fatty acids,

UI = unsaturation index = 1 × (% monoenoics) + 2 × (% dienoics) + 3 × (% trienoics) + 4 × (% tetraenoics) + 5 × (% pentaenoics) + 6 × (% hexaenoics).

RMSE = root-mean-square error. Ns= not significant, t= 0.1>*P*>0.05. Different letters in a line indicate significant differences ($P<0.05$).

Contrast 1: Females-Males; C2: VLBIW-(LBIW+MBIW+HBIW); C3: VLBIW-LBIW; C4: LBIW-(MBIW+HBIW); C5: MBIW-HBIW; Int: Interaction birth-Wt and sex.

# Anexo 3: Material suplementario Experimento 2

## Additional File 1.

**Reliability criteria for biochemical plasma assays.**

| | Glucose | Fructosamine | Triglycerides | Total cholesterol | HDL-c | LDL-c |
|---|---|---|---|---|---|---|
| **Kit reference** | 41011 | 1001158 | 41033 | 41021 | MI1001096 | MD41023 |
| **According to manufacturer's instructions** | | | | | | |
| Detection limit | 0.3709 mg/dL | 1 µmol/L | 0.000 mg/dL | 0.000 mg/dL | 3 mg/dL | 3.7 mg/dL |
| CV Intra-assay, % | 0.44 | 1.79 | 0.52 | 0.77 | 2.55 | 2.24 |
| CV Inter-assay , % | 2.78 | 1.99 | 3.45 | 2.54 | 4.16 | 3.97 |

HDL-c: high-density lipoprotein cholesterol, LDL-c: low-density lipoprotein cholesterol.

## Additional File 2.

**Supplementary Table 1. Calculated analysis (g/kg, dry-matter basis) and fatty acid composition of the diets.**

| | Sow | | Pig | | | | | |
|---|---|---|---|---|---|---|---|---|
| | Gestation | Lactation | 1st Prestarter | Prestarter | Starter | Growth 1 | Growth 2 | Finisher |
| **Age** | | | 7-25 days old | 26-35 days old | 36-70 days old | 71-140 days old | 141-220 days old | from 221 days old |
| **Calculated analysis**[1] | | | | | | | | |
| Net energy, Mcal/kg | 2217 | 2250 | 2300 | 2400 | 2400 | 2350 | 2400 | 2464 |
| Dry Matter (DM) | 899.9 | 894.9 | 894.5 | 895.4 | 895.6 | 894.5 | 899.4 | 906.1 |
| Crude protein | 130 | 157.5 | 150 | 157.8 | 150 | 151.9 | 142.5 | 125 |
| Crude fat | 26.1 | 36.6 | 40 | 40 | 40 | 31.5 | 42 | 57 |
| Crude fiber | 42.2 | 43.9 | 20 | 29.3 | 28.8 | 32.4 | 32.9 | 34 |
| Nitrogen-free extracts (NFE) | 638.5 | 593.2 | 634.5 | 621.6 | 628.9 | 629.1 | 633.7 | 643.3 |
| Ash | 63.1 | 63.7 | 50 | 46.7 | 47.9 | 49.6 | 48.3 | 46.8 |
| **Fatty acids composition, g/100 g total fatty acids** | | | | | | | | |
| C14:0 | 1.12 | 0.8 | 0.73 | 0.73 | 0.78 | 1.04 | 0.66 | 0.91 |
| C16:0 | 16.14 | 14.07 | 23.61 | 20.69 | 20.11 | 21.86 | 22.85 | 22.24 |
| C16:1 n-9 | 0.04 | 0.03 | 0.11 | 0.18 | 0.21 | 0.31 | 0.18 | 0.16 |
| C16:1 n-7 | 1.14 | 0.78 | 0.72 | 0.91 | 1.2 | 1.64 | 0.73 | 1.19 |
| C17:0 | 0.41 | 0.43 | 0.43 | 0.61 | 0.52 | 0.52 | 0.54 | 0.48 |
| C17:1 | 0.18 | 0.06 | 0.13 | 0.18 | 0.23 | 0.29 | 0.15 | 0.2 |
| C18:0 | 5.78 | 3.42 | 5.86 | 5.18 | 6.02 | 7.7 | 4.61 | 7.07 |
| C18:1 n-9 | 25.87 | 22.17 | 28.97 | 24.91 | 29.96 | 33.5 | 23.91 | 32.42 |
| C18:1 n-7 | 1.12 | 1.1 | 0.85 | 1.3 | 1.4 | 1.21 | 1.51 | 1.39 |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| C18:2 n-6 | 40.06 | 47.23 | 34.43 | 39.74 | 34.79 | 27.17 | 36.54 | 28.58 |
| C18:3 n-3 | 4.54 | 5.06 | 2.54 | 3.32 | 2.81 | 2.47 | 4.38 | 3.15 |
| C20:0 | 0.31 | 0.33 | 0.32 | 0.25 | 0.24 | 0.18 | 0.31 | 0.33 |
| C20:1 n-9 | 0.47 | 0.4 | 0.5 | 0.7 | 0.72 | 0.84 | 0.89 | 1.01 |
| C20:3n-6 | 0.16 | 0.13 | 0.11 | 0.09 | 0.13 | 0.17 | 0.06 | 0.11 |
| C20:4 n-6 | 0.02 | 0.11 | 0.04 | 0.26 | 0.05 | 0.08 | 0.19 | 0.05 |
| C20:5 n-3 | 0.91 | 1.34 | 0.02 | 0.25 | 0.19 | 0.1 | 0.15 | 0.2 |
| C22:4 n-6 | 0.05 | 0.18 | 0.29 | 0.15 | 0.12 | 0.25 | 0.29 | 0.1 |
| C22:5 n-3 | 0.2 | 0.18 | 0.1 | 0.18 | 0.18 | 0.43 | 1.63 | 0.15 |
| C22:6 n-3 | 1.46 | 2.19 | 0.24 | 0.38 | 0.32 | 0.25 | 0.4 | 0.27 |
| SFA | 23.77 | 19.05 | 30.93 | 27.45 | 27.68 | 31.3 | 28.97 | 31.02 |
| MUFA | 28.82 | 24.54 | 31.29 | 28.17 | 33.71 | 37.79 | 27.38 | 36.37 |
| PUFA | 47.41 | 56.41 | 37.77 | 44.38 | 38.6 | 30.9 | 43.65 | 32.61 |

[1]According to [21], g/kg of diet. NFE: DM – (ash + crude protein + crude fat + crude fiber). SFA = sum of saturated fatty acids, MUFA = sum of monounsaturated fatty acids, PUFA = sum of polyunsaturated fatty acids.

## Additional File 3.

**Supplementary Table 2: Phenotypic parameters at birth and weaning.**

| Variables | N | Control | | | | Underfed | | | | RMSE | *P*-value | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | LBW | | NBW | | LBW | | NBW | | | | | | | |
| | | Females | Males | Females | Males | Females | Males | Females | Males | | BW | Sex | Nutri | BW* Sex | BW* Nutri |
| **Birth** | | | | | | | | | | | | | | | |
| Body Weight, kg | 681 | 0.82 B | 0.78 B | 1.41 A | 1.43 A | 0.85 B | 0.80 B | 1.47 A | 1.47 A | 0.22 | <.0001 | ns | ns | ns | ns |
| Occipito-nasal Length, cm | 679 | 11.88 B | 11.65 BC | 12.69 A | 12.72 A | 11.43 C | 11.42 C | 12.40 A | 12.45 A | 0.60 | <.0001 | ns | <.0001 | ns | ns |
| Trunk Length, cm | 680 | 19.62 CD | 19.25 D | 23.01 B | 23.08 B | 20.25 C | 20.00 CD | 24.31 A | 24.03 A | 1.69 | <.0001 | ns | 0.0001 | ns | ns |
| Abdominal Perimeter, cm | 681 | 15.67 B | 15.10 B | 18.71 A | 18.83 A | 15.80 B | 15.15 B | 19.25 A | 19.45 A | 1.53 | <.0001 | ns | ns | t | ns |
| Thoracic Perimeter, cm | 681 | 20.24 B | 19.49 BC | 24.50 A | 24.39 A | 20.20 B | 19.15 C | 24.09 A | 24.03 A | 1.54 | <.0001 | 0.01 | t | 0.04 | ns |
| Biparietal Diameter, cm | 681 | 4.67 B | 4.64 B | 5.37 A | 5.37 A | 4.69 B | 4.68 B | 5.43 A | 5.45 A | 0.25 | <.0001 | ns | ns | ns | ns |
| Maximum Thoracic Diameter, cm | 681 | 5.55 C | 5.52 C | 6.95 A | 6.93 A | 5.87 B | 5.42 C | 7.23 A | 7.20 A | 0.56 | <.0001 | t | 0.04 | ns | ns |
| **Weaning** | | | | | | | | | | | | | | | |
| Body Weight, kg | 599 | 4.16 B | 4.16 B | 5.76 A | 5.74 A | 4.68 B | 4.64 B | 5.50 A | 5.46 A | 1.20 | <.0001 | ns | ns | ns | 0.04 |
| Occipito-nasal Length, cm | 600 | 15.19 CD | 15.11 D | 16.28 A | 16.30 A | 15.75 ABC | 15.53 BC D | 16.02 AB | 16.05 AB | 1.25 | 0.0003 | ns | ns | ns | 0.02 |
| Trunk Length, cm | 600 | 35.11 B | 34.83 B | 40.53 A | 39.90 A | 37.14 B | 37.22 B | 40.09 A | 39.72 A | 3.79 | <.0001 | ns | ns | ns | 0.03 |
| Abdominal Perimeter, cm | 600 | 30.48 C | 30.41 C | 34.62 A | 34.13 AB | 30.43 C | 30.67 C | 32.11 BC | 31.89 C | 3.47 | <.0001 | ns | 0.03 | ns | 0.02 |
| Thoracic Perimeter, cm | 600 | 35.17 C | 34.25 C | 39.09 A | 38.64 A | 36.00 BC | 35.94 BC | 38.34 A | 37.60 AB | 3.34 | <.0001 | ns | ns | ns | 0.04 |
| Backfat depth, cm | 600 | 0.42 | 0.44 | 0.45 | 0.49 | 0.49 | 0.45 | 0.41 | 0.44 | 0.11 | ns | ns | ns | ns | 0.02 |

| | | | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Loin diameter, cm | 600 | 0.90 | AB | 0.83 | ABC | 0.97 | A | 0.96 | A | 0.70 | C | 0.68 | C | 0.77 | BC | 0.75 | BC | 0.23 | 0.01 | ns | <.0001 | ns | ns |
| ADWG, kg/d | 600 | 0.13 | C | 0.14 | BC | 0.19 | A | 0.19 | A | 0.15 | BC | 0.15 | BC | 0.16 | AB | 0.16 | AB | 0.04 | <.0001 | ns | ns | ns | 0.005 |

BW= Birth weight, Nutri= Maternal Nutrition. LBW= Low birth-weight, NBW= Normal birth-weight. ADWG= Average daily weight gain. RMSE = root-mean-square error. Ns= not significant, t= 0.1>*P*>0.05. Different letters in a line indicate significant differences (*P*<0.05).

## Additional File 4.

**Supplementary Table 3: Growth during growing-fattening phase.**

| Variables | N | Control | | | | Underfed | | | | RMSE | *P*-value | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | LBW | | NBW | | LBW | | NBW | | | | | | | | |
| | | Females | Males | Females | Males | Females | Males | Females | Males | | BW | Sex | Nutri | BW* Sex | BW* Nutri | BW* Sex* Nutri |
| **110 days old** | | | | | | | | | | | | | | | | |
| Body Weight, kg | 468 | 36.6 AB | 34.2 ABC | 37.2 A | 36.4 AB | 28.7 D | 34.1 ABC | 31.3 CD | 32.9 BC | 5.29 | 0.02 | ns | ns | ns | ns | 0.002 |
| ADWG, kg/d | 469 | 0.38 A | 0.35 ABC | 0.37 AB | 0.36 AB | 0.30 C | 0.37 AB | 0.33 BC | 0.34 ABC | 0.06 | ns | ns | t | ns | ns | 0.001 |
| FCR, kg/kg | 468 | 0.96 C | 1.28 BC | 1.32 B | 1.54 B | 2.18 A | 1.22 BC | 1.57 B | 1.33 B | 0.48 | ns | ns | ns | t | 0.002 | <.0001 |
| **150 days old** | | | | | | | | | | | | | | | | |
| Body Weight, kg | 464 | 49.9 C | 57.3 AB | 58.3 A | 58.8 A | 51.8 BC | 58.6 A | 55.6 AB | 57.9 A | 8.14 | 0.001 | 0.006 | ns | t | ns | ns |
| ADWG, kg/d | 464 | 0.38 C | 0.66 A | 0.60 AB | 0.64 AB | 0.55 B | 0.59 AB | 0.58 AB | 0.60 AB | 0.13 | 0.002 | <.0001 | ns | 0.006 | 0.04 | 0.01 |
| FCR, kg/kg | 464 | 3.55 A | 2.05 B | 2.03 B | 1.96 B | 2.00 B | 1.91 B | 1.90 B | 2.04 B | 0.92 | 0.005 | 0.02 | 0.02 | 0.009 | 0.004 | 0.03 |
| **180 days old** | | | | | | | | | | | | | | | | |
| Body Weight, kg | 460 | 69.4 B | 83.0 A | 81.7 A | 84.2 A | 73.6 B | 86.3 A | 82.3 A | 87.5 A | 10.5 | <.0001 | <.0001 | 0.04 | 0.02 | ns | ns |
| ADWG, kg/d | 460 | 0.56 D | 0.73 AB | 0.67 BC | 0.73 AB | 0.59 CD | 0.71 AB | 0.69 ABC | 0.77 A | 0.13 | 0.004 | <.0001 | ns | 0.04 | ns | ns |
| FCR, kg/kg | 460 | 4.99 A | 4.12 BC | 3.67 C | 3.67 C | 4.82 A | 4.42 AB | 3.85 BC | 3.73 C | 0.83 | <.0001 | 0.01 | ns | 0.04 | ns | ns |
| **215 days old** | | | | | | | | | | | | | | | | |
| Body Weight, kg | 446 | 94.4 C | 114 AB | 113 AB | 115 A | 93.5 C | 105 B | 105 B | 110 AB | 12.7 | <.0001 | <.0001 | 0.0003 | 0.009 | ns | ns |

| | | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| ADWG, kg/d | 446 | 0.75 | B | 0.89 | A | 0.91 | A | 0.89 | A | 0.64 | CB | 0.61 | C | 0.73 | B | 0.72 | B | 0.15 | 0.0003 | ns | <.0001 | ns | ns | ns |
| FCR, kg/kg | 446 | 4.41 | CD | 4.19 | CD | 3.73 | D | 4.02 | D | 6.06 | A | 6.17 | A | 4.90 | BC | 5.24 | B | 1.06 | <.0001 | ns | <.0001 | ns | t | ns |
| Backfat depth, cm | 451 | 1.77 | C | 2.46 | AB | 2.18 | B | 2.35 | AB | 2.41 | AB | 2.30 | AB | 2.37 | AB | 2.64 | A | 0.46 | t | 0.002 | 0.008 | ns | ns | 0.02 |
| Outer layer | 451 | 0.97 | C | 1.14 | AB | 1.22 | A | 1.25 | A | 1.28 | A | 0.99 | BC | 1.28 | A | 1.23 | A | 0.23 | 0.0002 | ns | ns | ns | ns | 0.005 |
| Inner layer | 451 | 0.80 | D | 1.31 | AB | 0.96 | CD | 1.10 | BC | 1.14 | BC | 1.30 | AB | 1.09 | BC | 1.41 | A | 0.34 | ns | <.0001 | 0.002 | ns | ns | 0.006 |
| Loin diameter, cm | 450 | 2.84 | C | 3.37 | AB | 2.92 | BC | 3.50 | A | 2.60 | C | 3.74 | A | 2.95 | BC | 3.48 | A | 0.61 | ns | <.0001 | ns | ns | ns | ns |

BW= Birth weight, Nutri= Maternal Nutrition. LBW= Low birth-weight, NBW= Normal birth-weight. ADWG= Average daily weight gain, FCR= Feed conversion rate. RMSE = root-mean-square error. Ns= not significant, t= 0.1>*P*>0.05. Different letters in a line indicate significant differences (*P*<0.05).

## Additional File 5.

**Supplementary Table 4: Fatty acids composition of liver (g/100 g total fatty acids).**

| | | Neutral Lipids | | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | N | Control | | | | | | | | Underfed | | | | | | | | RMSE | *P*-value | | | |
| | | LBW | | | | NBW | | | | LBW | | | | NBW | | | | | | | | |
| | | Females | | Males | | Females | | Males | | Females | | Males | | Females | | Males | | | **BW** | **Sex** | **Nutri** | **BW* Sex* Nutri** |
| C14:0 | 343 | 0.62 | | 0.80 | | 0.78 | | 0.78 | | 0.71 | | 0.81 | | 0.80 | | 0.68 | | 0.26 | ns | ns | ns | ns |
| C16:0 | 343 | 19.21 | | 20.71 | | 20.38 | | 20.34 | | 20.78 | | 20.35 | | 20.76 | | 19.59 | | 2.42 | ns | ns | ns | t |
| C16:1 n-9 | 343 | 0.52 | | 0.63 | | 0.67 | | 0.67 | | 0.62 | | 0.69 | | 0.65 | | 0.59 | | 0.47 | ns | ns | ns | 0.04 |
| C16:1 n-7 | 343 | 1.33 | | 1.73 | | 1.58 | | 1.73 | | 1.39 | | 1.75 | | 1.61 | | 1.49 | | 0.20 | ns | t | ns | ns |
| C17:0 | 343 | 0.54 | ABCD | 0.58 | ABC | 0.62 | A | 0.59 | AB | 0.45 | CD | 0.41 | D | 0.47 | BCD | 0.47 | BCD | 0.12 | ns | ns | <.0001 | ns |
| C17:1 | 343 | 0.36 | A | 0.37 | A | 0.31 | AB | 0.35 | A | 0.22 | C | 0.26 | BC | 0.26 | BC | 0.26 | BC | 0.07 | ns | ns | <.0001 | t |
| C18:0 | 343 | 25.77 | | 23.16 | | 22.84 | | 22.19 | | 24.22 | | 22.25 | | 22.74 | | 23.53 | | 3.65 | ns | ns | ns | ns |
| C18:1 n-9 | 343 | 24.34 | | 28.65 | | 26.69 | | 27.76 | | 26.53 | | 28.06 | | 26.69 | | 25.28 | | 4.91 | ns | ns | ns | t |
| C18:1 n-7 | 343 | 1.59 | | 1.76 | | 1.65 | | 1.77 | | 1.69 | | 1.72 | | 1.69 | | 1.72 | | 0.27 | ns | ns | ns | ns |
| C18:2 n-6 | 343 | 10.94 | | 9.76 | | 11.02 | | 10.65 | | 10.95 | | 10.52 | | 10.89 | | 11.17 | | 1.56 | ns | ns | ns | ns |
| C18:3 n-3 | 343 | 0.21 | B | 0.24 | B | 0.27 | AB | 0.27 | AB | 0.28 | AB | 0.33 | AB | 0.38 | A | 0.37 | A | 0.11 | 0.04 | ns | 0.001 | ns |
| C20:0 | 343 | 0.35 | | 0.33 | | 0.34 | | 0.32 | | 0.19 | | 0.24 | | 0.27 | | 0.31 | | 0.24 | ns | ns | ns | ns |
| C20:1 n-9 | 343 | 0.72 | A | 0.71 | A A | 0.63 | A | 0.67 | A | 0.33 | B | 0.33 | B | 0.36 | B | 0.37 | B | 0.22 | ns | ns | <.0001 | ns |
| C20:3 n-6 | 343 | 0.67 | A | 0.47 | B | 0.60 | AB | 0.49 | AB | 0.40 | B | 0.39 | B | 0.41 | B | 0.44 | AB | 0.22 | ns | ns | 0.009 | 0.03 |
| C20:4 n-6 | 343 | 9.43 | | 7.62 | | 8.93 | | 8.73 | | 8.64 | | 9.17 | | 9.23 | | 10.54 | | 3.13 | ns | ns | ns | t |
| C20:5 n-3 | 343 | 0.24 | | 0.20 | | 0.23 | | 0.22 | | 0.15 | | 0.21 | | 0.21 | | 0.25 | | 0.09 | ns | ns | ns | t |

| | | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| C22:4 n-6 | 343 | 0.66 | | 0.56 | | 0.66 | | 0.66 | | 0.50 | | 0.55 | | 0.61 | | 0.71 | | 0.23 | ns | ns | ns | ns |
| C22:5 n-3 | 343 | 0.90 | | 0.77 | | 0.91 | | 0.87 | | 0.83 | | 0.87 | | 0.95 | | 1.06 | | 0.44 | ns | ns | ns | ns |
| C22:6 n-3 | 343 | 0.94 | | 0.95 | | 0.89 | | 0.93 | | 1.12 | | 1.06 | | 1.02 | | 1.19 | | 0.30 | ns | ns | 0.02 | ns |
| SFA | 343 | 46.69 | | 45.58 | | 45.03 | | 44.24 | | 46.35 | | 44.07 | | 45.04 | | 44.57 | | 2.66 | ns | t | ns | ns |
| MUFA | 343 | 28.87 | | 33.85 | | 31.47 | | 33.04 | | 30.77 | | 32.83 | | 31.27 | | 29.70 | | 5.75 | ns | ns | ns | t |
| PUFA | 343 | 23.99 | | 20.57 | | 23.50 | | 22.73 | | 22.88 | | 23.10 | | 23.69 | | 25.73 | | 5.56 | ns | ns | ns | t |
| Σn3 | 343 | 2.29 | | 2.15 | | 2.31 | | 2.30 | | 2.39 | | 2.47 | | 2.55 | | 2.87 | | 0.75 | ns | ns | ns | ns |
| Σn6 | 343 | 21.70 | | 18.41 | | 21.19 | | 20.42 | | 20.49 | | 20.63 | | 21.14 | | 22.86 | | 4.85 | ns | ns | ns | t |
| Σn6/Σn3 | 343 | 9.68 | A | 8.62 | ABC | 9.48 | AB | 9.21 | ABC | 8.80 | ABC | 8.61 | ABC | 8.42 | C | 8.16 | C | 1.05 | ns | t | 0.004 | ns |
| UI | 343 | 1.05 | | 0.99 | | 1.06 | | 1.05 | | 1.03 | | 1.07 | | 1.07 | | 1.13 | | 0.15 | ns | ns | ns | t |
| MUFA/SFA | 343 | 0.62 | | 0.75 | | 0.71 | | 0.76 | | 0.67 | | 0.75 | | 0.70 | | 0.67 | | 0.15 | ns | ns | ns | t |
| C18:1/C18:0 | 343 | 1.07 | | 1.35 | | 1.33 | | 1.45 | | 1.20 | | 1.37 | | 1.29 | | 1.21 | | 0.48 | ns | ns | ns | ns |

**Polar Lipids**

| | N | Control | | | | | | | | Underfed | | | | | | | | RMSE | *P*-value | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | LBW | | | | NBW | | | | LBW | | | | NBW | | | | | | | | | |
| | | Females | | Males | | Females | | Males | | Females | | Males | | Females | | Males | | | **BW** | **Sex** | **Nutri** | **BW* Sex** | **BW* Sex* Nutri** |
| C14:0 | 344 | 0.22 | | 0.34 | | 0.28 | | 0.30 | | 0.34 | | 0.26 | | 0.36 | | 0.34 | | 0.13 | ns | ns | ns | ns | ns |
| C16:0 | 344 | 15.94 | B | 18.57 | A A | 17.90 | AB | 18.38 | A | 18.34 | A | 17.29 | AB | 18.52 | A | 18.23 | A | 1.96 | ns | ns | ns | ns | 0.04 |
| C16:1 n-9 | 344 | 0.20 | B | 0.26 | B | 0.25 | AB | 0.25 | AB | 0.34 | A | 0.26 | AB | 0.33 | A | 0.32 | A | 0.19 | ns | ns | 0.004 | ns | ns |
| C16:1 n-7 | 344 | 0.57 | | 0.76 | | 0.66 | | 0.72 | | 0.71 | | 0.72 | | 0.77 | | 0.77 | | 0.11 | ns | ns | ns | ns | ns |
| C17:0 | 344 | 0.59 | ABC | 0.68 | ABC | 0.71 | A | 0.70 | AB | 0.54 | BC | 0.53 | C | 0.60 | ABC | 0.57 | ABC | 0.14 | t | ns | 0.003 | ns | ns |
| C17:1 | 344 | 0.16 | C | 0.32 | A | 0.23 | BC | 0.24 | B | 0.18 | BC | 0.24 | BC | 0.20 | BC | 0.20 | BC | 0.07 | ns | 0.003 | t | 0.004 | ns |
| C18:0 | 344 | 32.29 | A | 29.72 | B | 30.12 | B | 29.60 | B | 30.70 | AB | 30.64 | AB | 29.52 | B | 28.98 | B | 2.01 | 0.01 | t | ns | ns | ns |

| | | | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| C18:1 n-9 | 344 | 15.30 | B | 17.88 | A | 16.01 | AB | 16.66 | AB | 17.87 | A | 17.67 | A | 17.77 | A | 17.68 | A | 2.06 | ns | ns | 0.01 | ns | ns |
| C18:1 n-7 | 344 | 1.27 | B | 1.64 | A | 1.38 | AB | 1.53 | A | 1.53 | A | 1.56 | A | 1.55 | A | 1.61 | A | 0.24 | ns | 0.01 | t | ns | t |
| C18:2 n-6 | 344 | 13.07 | A | 11.49 | B | 12.22 | AB | 11.70 | B | 12.10 | AB | 11.68 | B | 11.76 | B | 11.73 | B | 1.07 | ns | 0.02 | ns | ns | t |
| C18:3 n-3 | 344 | 0.15 | C | 0.25 | A B | 0.20 | BC | 0.22 | AB | 0.23 | AB | 0.24 | AB | 0.26 | AB | 0.27 | A | 0.06 | ns | 0.04 | 0.003 | ns | ns |
| C20:0 | 344 | 0.28 | AB | 0.33 | A | 0.32 | A | 0.35 | A | 0.16 | B | 0.23 | AB | 0.27 | AB | 0.30 | AB | 0.13 | t | ns | 0.02 | ns | ns |
| C20:1 n-9 | 344 | 0.22 | B | 0.31 | A A | 0.25 | AB | 0.26 | AB | 0.22 | B | 0.18 | B | 0.22 | B | 0.21 | B | 0.08 | ns | ns | 0.007 | ns | t |
| C20:3 n-6 | 344 | 0.86 | A | 0.67 | B | 0.77 | A | 0.65 | AB | 0.47 | B | 0.49 | B | 0.51 | B | 0.49 | B | 0.22 | ns | ns | <.0001 | ns | t |
| C20:4 n-6 | 344 | 14.58 | | 12.78 | | 14.43 | | 14.10 | | 12.52 | | 13.98 | | 13.30 | | 13.93 | | 2.61 | ns | ns | ns | ns | ns |
| C20:5 n-3 | 344 | 0.44 | | 0.36 | A | 0.38 | | 0.40 | | 0.41 | | 0.41 | | 0.38 | | 0.42 | | 0.10 | ns | ns | ns | ns | ns |
| C22:4 n-6 | 344 | 0.78 | AB | 0.80 | B | 0.88 | A | 0.89 | A | 0.60 | B | 0.59 | B | 0.70 | AB | 0.76 | AB | 0.21 | 0.03 | ns | 0.0009 | ns | ns |
| C22:5 n-3 | 344 | 1.46 | | 1.37 | | 1.64 | | 1.57 | | 1.28 | | 1.36 | | 1.43 | | 1.46 | | 0.46 | ns | ns | ns | ns | ns |
| C22:6 n-3 | 344 | 1.63 | | 1.47 | | 1.36 | | 1.48 | | 1.45 | | 1.67 | | 1.53 | | 1.73 | | 0.44 | ns | ns | ns | ns | ns |
| SFA | 344 | 49.32 | | 49.64 | | 49.34 | | 49.32 | | 50.09 | | 48.95 | | 49.27 | | 48.42 | | 2.27 | ns | ns | ns | ns | ns |
| MUFA | 344 | 17.71 | B | 21.18 | A | 18.79 | AB | 19.67 | AB | 20.85 | A | 20.63 | A | 20.86 | A | 20.79 | A | 2.50 | ns | ns | 0.02 | ns | t |
| PUFA | 344 | 32.97 | | 29.18 | | 31.88 | | 31.01 | | 29.06 | | 30.42 | | 29.88 | | 30.79 | | 3.94 | ns | ns | ns | ns | t |
| Σn3 | 344 | 3.68 | | 3.44 | | 3.58 | | 3.66 | | 3.36 | | 3.68 | | 3.61 | | 3.88 | | 0.76 | ns | ns | ns | ns | ns |
| Σn6 | 344 | 29.29 | | 25.74 | | 28.30 | | 27.35 | | 25.70 | | 26.74 | | 26.27 | | 26.91 | | 3.36 | ns | ns | ns | ns | t |
| Σn6/Σn3 | 344 | 7.99 | | 7.60 | | 8.11 | | 7.71 | | 7.80 | | 7.51 | | 7.52 | | 7.05 | | 1.21 | ns | ns | ns | ns | ns |
| UI | 344 | 1.28 | | 1.19 | | 1.26 | | 1.24 | | 1.17 | | 1.23 | | 1.21 | | 1.25 | | 0.13 | ns | ns | ns | ns | ns |
| MUFA/SFA | 344 | 0.36 | B | 0.43 | A | 0.38 | AB | 0.40 | AB | 0.42 | A | 0.42 | A | 0.42 | A | 0.43 | A | 0.05 | ns | t | 0.007 | ns | ns |
| C18:1/C18:0 | 344 | 0.51 | B | 0.66 | A | 0.58 | AB | 0.62 | AB | 0.64 | A | 0.63 | A | 0.66 | A | 0.67 | A | 0.10 | ns | t | 0.02 | ns | ns |

BW= Birth weight, Nutri= Maternal Nutrition. LBW= Low birth-weight, NBW= Normal birth-weight. SFA = sum of saturated fatty acids, MUFA = sum of monounsaturated fatty acids, PUFA = sum of polyunsaturated fatty acids, UI = unsaturation index = 1 × (% monoenoics) + 2 × (% dienoics) + 3 × (% trienoics) + 4 × (% tetraenoics) + 5 × (% pentaenoics) + 6 × (% hexaenoics). RMSE = root-mean-square error. Ns= not significant, t= 0.1>P>0.05. Different letters in a line indicate significant differences (P<0.05).

## Additional File 6.

**Supplementary Table 5: Fatty acids composition of longissimus dorsi muscle (g/100 g total fatty acids).**

| | | Neutral Lipids | | | | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | N | Control | | | | | | | | Underfed | | | | | | | | RMSE | *P*-value | | | | |
| | | LBW | | | | NBW | | | | LBW | | | | NBW | | | | | | | | | |
| | | Females | | Males | | Females | | Males | | Females | | Males | | Females | | Males | | | **Sex** | **Nutri** | | | |
| C14:0 | 377 | 1.54 | | 1.51 | | 1.48 | | 1.48 | | 1.48 | | 1.56 | | 1.53 | | 1.55 | | 0.15 | ns | ns | | | |
| C16:0 | 377 | 25.5 | | 24.4 | | 25.4 | | 24.9 | | 25.4 | | 25.5 | | 25.3 | | 25.2 | | 2.49 | ns | ns | | | |
| C16:1 n-9 | 377 | 0.20 | ABC | 0.20 | AB | 0.21 | A | 0.21 | A | 0.16 | D | 0.17 | CD | 0.17 | BCD | 0.17 | CD | 0.74 | ns | <.0001 | | | |
| C16:1 n-7 | 377 | 4.40 | AB | 4.67 | AB | 4.41 | AB | 4.68 | AB | 4.17 | B | 4.61 | AB | 4.65 | AB | 4.79 | A | 0.04 | 0.04 | ns | | | |
| C17:0 | 377 | 0.14 | | 0.15 | | 0.12 | | 0.15 | | 0.12 | | 0.12 | | 0.12 | | 0.13 | | 0.04 | ns | 0.04 | | | |
| C17:1 | 377 | 0.18 | | 0.19 | | 0.16 | | 0.19 | | 0.16 | | 0.16 | | 0.16 | | 0.18 | | 0.06 | ns | ns | | | |
| C18:0 | 377 | 11.2 | | 10.8 | | 10.9 | | 10.7 | | 11.0 | | 10.8 | | 10.6 | | 10.6 | | 1.21 | ns | ns | | | |
| C18:1 n-9 | 377 | 47.5 | C | 48.4 | BC | 47.0 | C | 48.1 | BC | 50.3 | A | 49.1 | ABC | 49.5 | AB | 49.1 | ABC | 3.24 | ns | <.0001 | | | |
| C18:1 n-7 | 377 | 4.66 | A | 4.91 | A | 4.70 | A | 4.94 | A | 3.01 | C | 3.41 | BC | 3.47 | B | 3.53 | B | 0.62 | 0.04 | <.0001 | | | |
| C18:2 n-6 | 377 | 2.62 | | 2.68 | | 2.44 | | 2.70 | | 2.30 | | 2.44 | | 2.56 | | 2.54 | | 0.54 | ns | ns | | | |
| C18:3 n-3 | 377 | 0.44 | D | 0.47 | ABCD | 0.44 | CD | 0.46 | BCD | 0.48 | ABC | 0.49 | AB | 0.50 | A | 0.50 | A | 0.05 | t | <.0001 | | | |
| C20:0 | 377 | 0.20 | A | 0.19 | AB | 0.17 | AB | 0.19 | AB | 0.16 | B | 0.17 | B | 0.16 | B | 0.17 | B | 0.04 | ns | 0.003 | | | |
| C20:1 n-9 | 377 | 0.97 | | 0.97 | | 0.91 | | 0.94 | | 0.97 | | 0.98 | | 0.96 | | 0.99 | | 0.12 | ns | ns | | | |
| C20:3 n-6 | 377 | nd | | nd | | nd | | nd | | nd | | nd | | nd | | nd | | - | - | - | | | |
| C20:4 n-6 | 377 | 0.15 | A | 0.15 | AB | 0.14 | AB | 0.14 | AB | 0.11 | B | 0.12 | AB | 0.15 | AB | 0.13 | AB | 0.05 | ns | 0.03 | | | |
| C20:5 n-3 | 377 | nd | | nd | | nd | | nd | | nd | | nd | | nd | | nd | | - | - | - | | | |

| | | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| C22:4 n-6 | 377 | nd | | nd | | nd | | nd | | nd | | nd | | nd | | - | - | - | |
| C22:5 n-3 | 377 | 0.09 | | 0.10 | | 0.10 | | 0.11 | | 0.09 | | 0.08 | | 0.10 | | 0.10 | | 0.05 | ns | ns | |
| C22:6 n-3 | 377 | 0.06 | AB | 0.07 | AB | 0.07 | AB | 0.07 | AB | 0.05 | B | 0.09 | A | 0.06 | AB | 0.05 | B | 0.04 | ns | ns | |
| SFA | 377 | 38.6 | | 37.1 | | 38.6 | | 37.4 | | 38.1 | | 38.2 | | 37.7 | | 37.8 | | 3.07 | ns | ns | |
| MUFA | 377 | 57.9 | | 59.4 | | 58.1 | | 59.0 | | 58.8 | | 58.5 | | 58.9 | | 58.8 | | 2.90 | ns | ns | |
| PUFA | 377 | 3.37 | | 3.46 | | 3.23 | | 3.49 | | 3.03 | | 3.22 | | 3.36 | | 3.32 | | 0.60 | ns | ns | |
| Σn3 | 377 | 0.59 | | 0.64 | | 0.62 | | 0.64 | | 0.62 | | 0.66 | | 0.65 | | 0.65 | | 0.09 | ns | ns | |
| Σn6 | 377 | 2.78 | | 2.82 | | 2.61 | | 2.84 | | 2.41 | | 2.56 | | 2.70 | | 2.66 | | 0.55 | ns | t | |
| Σn6/Σn3 | 377 | 4.68 | A | 4.52 | AB | 4.29 | AB | 4.45 | AB | 3.86 | B | 3.94 | B | 4.15 | AB | 4.10 | AB | 0.77 | ns | 0.001 | |
| UI | 377 | 65.9 | | 67.6 | | 65.9 | | 67.3 | | 66.0 | | 66.2 | | 66.9 | | 66.7 | | 3.42 | ns | ns | |
| MUFA/SFA | 377 | 1.51 | | 1.64 | | 1.51 | | 1.61 | | 1.54 | | 1.55 | | 1.57 | | 1.58 | | 0.23 | ns | ns | |
| C18:1/C18:0 | 377 | 4.72 | | 5.05 | | 4.78 | | 5.04 | | 4.85 | | 4.93 | | 5.05 | | 5.02 | | 0.76 | ns | ns | |

**Polar Lipids**

| | N | Control | | | | | | | | Underfed | | | | | | | | RMSE | *P*-value | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | LBW | | | | NBW | | | | LBW | | | | NBW | | | | | | | | |
| | | Females | | Males | | Females | | Males | | Females | | Males | | Females | | Males | | | **BW** | **Sex** | **Nutri** | **BW* Nutri** |
| C14:0 | 377 | 2.09 | D | 2.89 | C | 2.78 | C | 2.65 | CD | 4.09 | AB | 3.61 | B | 4.26 | A | 3.99 | AB | 0.79 | ns | ns | <.0001 | ns |
| C16:0 | 377 | 19.6 | AB | 18.5 | CD | 18.8 | BCD | 18.3 | D | 20.0 | AB | 18.6 | AB | 19.8 | A | 19.4 | ABC | 1.42 | t | ns | 0.0001 | ns |
| C16:1 n-9 | 377 | 0.32 | C | 0.31 | C | 0.32 | C | 0.32 | C | 1.35 | AB | 1.19 | B | 1.45 | A | 1.37 | AB | 0.25 | ns | ns | <.0001 | ns |
| C16:1 n-7 | 377 | 1.24 | AB | 1.34 | A | 1.04 | B | 1.15 | AB | 1.10 | B | 1.24 | AB | 1.18 | AB | 1.25 | AB | 0.25 | ns | 0.03 | ns | 0.01 |
| C17:0 | 377 | 0.50 | AB | 0.58 | A | 0.50 | AB | 0.54 | AB | 0.46 | B | 0.52 | AB | 0.47 | B | 0.48 | B | 0.11 | ns | 0.02 | 0.02 | ns |
| C17:1 | 377 | 0.84 | AB | 0.70 | B | 0.90 | AB | 0.92 | AB | 1.07 | AB | 1.59 | A | 1.01 | AB | 1.27 | AB | 0.86 | ns | ns | 0.020 | ns |

| | | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| C18:0 | 377 | 9.59 | A | 9.20 | AB | 8.90 | BC | 8.96 | ABC | 7.99 | D | 8.53 | BCD | 8.51 | CD | 8.63 | BCD | 0.84 | ns | ns | <.0001 | 0.01 |
| C18:1 n-9 | 377 | 16.9 | A | 16.8 | A | 14.7 | BC | 15.8 | AB | 13.3 | C | 13.9 | C | 13.4 | C | 13.6 | C | 1.92 | 0.01 | ns | <.0001 | 0.03 |
| C18:1 n-7 | 377 | 3.48 | | 3.65 | | 3.47 | | 3.48 | | 3.34 | | 3.46 | | 3.43 | | 3.56 | | 0.46 | ns | ns | ns | ns |
| C18:2 n-6 | 377 | 27.8 | BC | 28.4 | ABC | 29.8 | A | 29.4 | AB | 28.7 | ABC | 27.6 | C | 27.9 | BC | 28.2 | ABC | 2.50 | t | ns | 0.04 | 0.03 |
| C18:3 n-3 | 377 | 0.54 | | 0.57 | | 0.54 | | 0.55 | | 0.54 | | 0.53 | | 0.52 | | 0.54 | | 0.08 | ns | ns | ns | ns |
| C20:0 | 377 | 0.22 | A | 0.22 | A | 0.22 | A | 0.24 | A | 0.16 | B | 0.17 | B | 0.17 | B | 0.17 | B | 0.05 | ns | ns | <.0001 | ns |
| C20:1 n-9 | 377 | 0.38 | A | 0.38 | A | 0.31 | BC | 0.34 | AB | 0.25 | D | 0.27 | CD | 0.26 | CD | 0.26 | CD | 0.08 | ns | ns | <.0001 | t |
| C20:3 n-6 | 377 | 1.28 | | 1.33 | | 1.34 | | 1.32 | | 1.30 | | 1.32 | | 1.25 | | 1.28 | | 0.16 | ns | ns | ns | ns |
| C20:4 n-6 | 377 | 11.1 | | 10.9 | | 11.9 | | 11.4 | | 12.1 | | 12.2 | | 12.2 | | 11.8 | | 1.73 | ns | ns | ns | ns |
| C20:5 n-3 | 377 | 0.47 | | 0.50 | | 0.50 | | 0.50 | | 0.52 | | 0.52 | | 0.49 | | 0.50 | | 0.10 | ns | ns | ns | ns |
| C22:4 n-6 | 377 | 1.26 | B | 1.26 | B | 1.45 | A | 1.40 | AB | 1.39 | AB | 1.31 | AB | 1.48 | A | 1.43 | AB | 0.22 | 0.001 | ns | ns | ns |
| C22:5 n-3 | 377 | 1.36 | B | 1.51 | AB | 1.64 | A | 1.62 | A | 1.52 | AB | 1.54 | AB | 1.49 | AB | 1.45 | AB | 0.27 | ns | ns | ns | 0.01 |
| C22:6 n-3 | 377 | 0.87 | A | 0.88 | A | 0.93 | A | 0.94 | A | 0.56 | C | 0.68 | B | 0.64 | BC | 0.64 | BC | 0.15 | ns | ns | <.0001 | ns |
| SFA | 377 | 32.0 | ABC | 31.4 | BC | 31.0 | C | 30.7 | C | 32.7 | A | 32.5 | AB | 33.2 | A | 32.6 | AB | 1.77 | ns | ns | <.0001 | t |
| MUFA | 377 | 23.1 | A | 23.1 | A | 20.7 | B | 21.9 | AB | 20.4 | B | 21.7 | AB | 20.7 | B | 21.3 | AB | 2.34 | 0.03 | t | 0.004 | 0.03 |
| PUFA | 377 | 44.8 | C | 45.4 | BC | 48.1 | A | 47.3 | AB | 46.7 | ABC | 45.7 | ABC | 46.0 | ABC | 45.9 | ABC | 3.15 | 0.03 | ns | ns | 0.008 |
| Σn3 | 377 | 3.25 | BC | 3.44 | AB | 3.61 | A | 3.60 | A | 3.13 | C | 3.26 | BC | 3.14 | C | 3.13 | C | 0.40 | ns | ns | <.0001 | 0.02 |
| Σn6 | 377 | 41.5 | B | 41.9 | B | 44.5 | A | 43.7 | AB | 43.6 | AB | 42.5 | AB | 42.8 | AB | 42.8 | AB | 3.04 | 0.04 | ns | ns | 0.01 |
| Σn6/Σn3 | 377 | 12.8 | BC | 12.2 | C | 12.8 | C | 12.2 | C | 13.9 | A | 13.1 | ABC | 13.7 | AB | 13.7 | AB | 1.27 | ns | t | <.0001 | ns |
| UI | 377 | 1.48 | B | 1.50 | AB | 1.56 | A | 1.54 | AB | 1.51 | AB | 1.51 | AB | 1.51 | AB | 1.50 | AB | 0.08 | t | ns | ns | 0.02 |
| MUFA/SFA | 379 | 0.72 | AB | 0.74 | A | 0.67 | BCD | 0.72 | ABC | 0.62 | D | 0.67 | ABCD | 0.63 | D | 0.66 | CD | 0.08 | ns | 0.02 | <.0001 | ns |
| C18:1/C18:0 | 379 | 2.12 | AB | 2.23 | A | 2.06 | AB | 2.18 | AB | 2.08 | AB | 2.06 | AB | 1.99 | B | 2.01 | AB | 0.27 | ns | ns | 0.03 | ns |

BW= Birth weight, Nutri= Maternal Nutrition. LBW= Low birth-weight, NBW= Normal birth-weight. SFA = sum of saturated fatty acids, MUFA = sum of monounsaturated fatty acids, PUFA = sum of polyunsaturated fatty acids, UI = unsaturation index = 1 × (% monoenoics) + 2 × (% dienoics) + 3 × (% trienoics) + 4 × (% tetraenoics) + 5 × (% pentaenoics) + 6 × (% hexaenoics). RMSE = root-mean-square error. Ns= not significant, t= 0.1>*P*>0.05. Different letters in a line indicate significant differences (*P*<0.05).

## Additional File 7.

**Supplementary Table 6: Fatty acids composition of subcutaneous fat (g/100 g total fatty acids).**

| | | Outer layer | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | N | Control | | | | Underfed | | | | RMSE | *P*-value | | | | |
| | | LBW | | NBW | | LBW | | NBW | | | | | | | |
| | | Females | Males | Females | Males | Females | Males | Females | Males | | Sex | Nutri | BW* Nutri | BW* Sex* Nutri |
| C14:0 | 391 | 1.58 A | 1.54 A | 1.54 A | 1.50 AB | 1.42 B | 1.50 AB | 1.49 AB | 1.52 A | 0.11 | ns | 0.007 | t | 0.002 |
| C16:0 | 391 | 24.8 A | 24.1 AB | 24.9 A | 24.1 AB | 23.3 BC | 23.1 C | 23.8 BC | 23.5 BC | 1.10 | 0.03 | <.0001 | ns | t |
| C16:1 n-9 | 391 | 0.30 | 0.36 | 0.35 | 0.36 | 0.38 | 0.30 | 0.31 | 0.30 | 0.40 | ns | ns | ns | ns |
| C16:1 n-7 | 391 | 2.73 | 2.78 | 2.78 | 2.94 | 2.75 | 2.94 | 2.81 | 2.91 | 0.12 | t | ns | ns | ns |
| C17:0 | 391 | 0.27 C | 0.31 AB | 0.28 BC | 0.29 ABC | 0.32 A | 0.28 BC | 0.28 BC | 0.30 ABC | 0.04 | ns | ns | ns | 0.02 |
| C17:1 | 391 | 0.31 B | 0.34 AB | 0.31 B | 0.34 AB | 0.36 A | 0.34 AB | 0.32 AB | 0.35 AB | 0.05 | ns | ns | ns | ns |
| C18:0 | 391 | 11.3 A | 11.4 A | 11.6 A | 10.9 AB | 10.7 AB | 10.3 B | 11.1 AB | 10.8 B | 1.05 | ns | 0.004 | ns | ns |
| C18:1 n-9 | 391 | 44.6 AB | 45.3 A | 44.2 AB | 45.2 A | 43.4 B | 44.8 A | 44.2 AB | 44.9 A | 1.53 | 0.003 | ns | ns | ns |
| C18:1 n-7 | 391 | 3.46 BC | 3.34 C | 3.28 C | 3.46 BC | 5.61 A | 5.33 A | 4.67 AB | 4.45 AB | 1.65 | ns | <.0001 | ns | ns |
| C18:2 n-6 | 391 | 8.49 B | 8.23 B | 8.61 B | 8.59 B | 9.38 A | 8.85 AB | 8.83 AB | 8.59 B | 0.74 | t | 0.002 | 0.02 | ns |
| C18:3 n-3 | 391 | 0.63 B | 0.63 B | 0.65 AB | 0.66 AB | 0.69 A | 0.65 AB | 0.65 AB | 0.64 AB | 0.06 | ns | ns | t | ns |
| C20:0 | 391 | 0.20 AB | 0.22 A | 0.19 AB | 0.19 AB | 0.21 A | 0.14 B | 0.16 AB | 0.19 AB | 0.08 | ns | t | ns | ns |
| C20:1 n-9 | 391 | 1.09 B | 1.22 A | 1.05 B | 1.12 AB | 1.09 B | 1.14 AB | 1.09 B | 1.17 AB | 0.14 | 0.002 | ns | ns | ns |
| C20:4 n-6 | 391 | 0.15 B | 0.16 AB | 0.16 AB | 0.16 AB | 0.19 A | 0.18 AB | 0.19 A | 0.19 A | 0.04 | ns | 0.0002 | ns | ns |
| SFA | 391 | 38.1 AB | 37.6 ABC | 38.5 A | 37.1 ABCD | 36.0 CD | 35.3 D | 36.8 BCD | 36.4 CD | 1.98 | 0.040 | <.0001 | ns | t |

| | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| MUFA | 391 | 52.5 | CD | 53.3 | BCD | 52.0 | D | 53.4 | ABCD | 53.6 | ABC | 54.9 | A | 53.4 | ABCD | 54.1 | AB | 1.7 | 0.002 | 0.0004 | ns | ns |
| PUFA | 391 | 9.27 | B | 9.03 | B | 9.42 | B | 9.41 | B | 10.2 | A | 9.68 | AB | 9.68 | AB | 9.42 | B | 0.81 | t | 0.002 | 0.02 | ns |
| Σn3 | 390 | 0.63 | B | 0.63 | B | 0.65 | B | 0.66 | AB | 0.70 | A | 0.65 | B | 0.66 | AB | 0.64 | B | 0.06 | ns | ns | 0.02 | ns |
| Σn6 | 391 | 8.63 | B | 8.39 | B | 8.77 | B | 8.75 | B | 9.56 | A | 9.03 | AB | 9.02 | AB | 8.78 | B | 1.11 | t | 0.001 | 0.02 | ns |
| Σn6/Σn3 | 390 | 13.6 | AB | 13.3 | B | 13.4 | AB | 13.2 | B | 13.5 | AB | 13.9 | A | 13.7 | AB | 13.7 | AB | 0.7 | ns | 0.02 | ns | ns |
| UI | 391 | 0.72 | CD | 0.72 | CD | 0.72 | D | 0.73 | BCD | 0.76 | A | 0.75 | AB | 0.74 | ABCD | 0.74 | ABC | 0.03 | ns | <.0001 | 0.04 | t |
| MUFA/SFA | 391 | 1.38 | C | 1.43 | BC | 1.36 | C | 1.45 | BC | 1.50 | AB | 1.56 | A | 1.46 | ABC | 1.49 | AB | 0.13 | 0.01 | <.0001 | ns | ns |
| C18:1/C18:0 | 391 | 4.29 | B | 4.36 | B | 4.14 | B | 4.51 | AB | 4.59 | AB | 4.92 | A | 4.45 | AB | 4.62 | AB | 0.57 | 0.03 | 0.003 | ns | ns |

**Inner layer**

| | N | Control | | | | | | | | Underfed | | | | | | | | RMSE | *P*-value | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | LBW | | | | NBW | | | | LBW | | | | NBW | | | | | | | | |
| | | Females | | Males | | Females | | Males | | Females | | Males | | Females | | Males | | | **BW** | **Sex** | **Nutri** | **BW* Sex* Nutri** |
| C14:0 | 393 | 1.54 | AB | 1.46 | B | 1.51 | AB | 1.46 | AB | 1.56 | AB | 1.58 | A | 1.56 | AB | 1.57 | AB | 0.14 | ns | ns | 0.004 | ns |
| C16:0 | 393 | 25.9 | A | 24.4 | B | 25.9 | A | 25.2 | AB | 25.5 | A | 25.4 | AB | 25.3 | AB | 25.0 | AB | 1.21 | ns | 0.004 | ns | t |
| C16:1 n-9 | 393 | 0.24 | | 0.26 | | 0.26 | | 0.26 | | 0.29 | | 0.29 | | 0.26 | | 0.27 | | 0.44 | ns | ns | t | ns |
| C16:1 n-7 | 393 | 2.43 | B | 2.56 | AB | 2.40 | B | 2.63 | AB | 2.41 | B | 2.66 | AB | 2.64 | AB | 2.84 | A | 0.07 | ns | 0.02 | ns | ns |
| C17:0 | 393 | 0.23 | C | 0.28 | AB | 0.24 | BC | 0.26 | ABC | 0.27 | AB | 0.26 | ABC | 0.25 | ABC | 0.28 | A | 0.04 | ns | 0.02 | t | t |
| C17:1 | 393 | 0.24 | B | 0.29 | AB | 0.24 | B | 0.27 | AB | 0.32 | A | 0.29 | AB | 0.31 | A | 0.32 | A | 0.07 | ns | ns | 0.0002 | ns |
| C18:0 | 393 | 13.2 | AB | 12.6 | ABC | 13.6 | A | 12.4 | ABC | 12.8 | ABC | 12.4 | BC | 12.5 | ABC | 12.0 | C | 1.36 | ns | 0.01 | 0.03 | t |
| C18:1 n-9 | 393 | 44.0 | B | 45.5 | A | 43.7 | B | 44.9 | AB | 44.4 | AB | 43.9 | B | 44.3 | AB | 44.8 | AB | 0.71 | ns | 0.02 | ns | 0.02 |

| | | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| C18:1 n-7 | 393 | 2.65 | D | 2.71 | CD | 2.57 | D | 2.82 | BCD | 3.00 | ABC D | 3.54 | A | 3.29 | ABC | 3.31 | AB | 1.63 | ns | ns | <.0001 | ns |
| C18:2 n-6 | 393 | 6.99 | | 7.25 | | 7.13 | | 7.22 | | 7.04 | | 7.41 | | 7.35 | | 7.32 | | 0.63 | ns | ns | ns | ns |
| C18:3 n-3 | 393 | 0.81 | AB | 0.85 | A | 0.84 | A | 0.85 | A | 0.76 | ABC | 0.72 | BC | 0.69 | C | 0.69 | C | 0.14 | ns | ns | <.0001 | ns |
| C20:0 | 393 | 0.21 | A | 0.23 | A | 0.21 | A | 0.21 | A | 0.14 | B | 0.13 | B | 0.14 | B | 0.14 | B | 0.05 | ns | ns | <.0001 | ns |
| C20:1 n-9 | 393 | 1.26 | | 1.26 | | 1.15 | | 1.17 | | 1.17 | | 1.17 | | 1.13 | | 1.14 | | 0.16 | 0.03 | ns | t | ns |
| C20:4 n-6 | 393 | 0.12 | C | 0.13 | BC | 0.14 | BC | 0.14 | BC | 0.16 | A | 0.14 | BC | 0.15 | AB | 0.15 | AB | 0.03 | ns | ns | 0.0009 | ns |
| SFA | 393 | 41.2 | A | 39.1 | B | 41.5 | A | 39.6 | AB | 40.3 | AB | 39.7 | AB | 39.8 | AB | 39.1 | B | 2.23 | ns | 0.002 | ns | 0.03 |
| MUFA | 393 | 50.8 | BC | 52.6 | A | 50.3 | C | 52.1 | AB | 51.6 | ABC | 51.9 | ABC | 52.0 | AB | 52.7 | A | 1.97 | ns | 0.003 | ns | 0.03 |
| PUFA | 393 | 7.92 | | 8.24 | | 8.11 | | 8.21 | | 7.96 | | 8.27 | | 8.19 | | 8.15 | | 0.68 | ns | ns | ns | ns |
| Σn3 | 392 | 0.81 | AB | 0.85 | A | 0.84 | A | 0.85 | A | 0.76 | ABC | 0.72 | BC | 0.69 | C | 0.69 | C | 0.14 | ns | ns | <.0001 | ns |
| Σn6 | 393 | 7.11 | | 7.39 | | 7.27 | | 7.36 | | 7.21 | | 7.55 | | 7.50 | | 7.46 | | 0.64 | ns | ns | ns | ns |
| Σn6/Σn3 | 392 | 8.71 | B | 8.64 | B | 8.65 | B | 8.61 | B | 9.51 | AB | 11.46 | A | 11.81 | A | 11.90 | A | 3.11 | ns | ns | <.0001 | ns |
| UI | 393 | 0.68 | BC | 0.70 | A | 0.68 | C | 0.70 | ABC | 0.69 | ABC | 0.69 | ABC | 0.69 | ABC | 0.70 | AB | 0.03 | ns | 0.004 | ns | 0.04 |
| MUFA/SFA | 393 | 1.23 | B | 1.36 | A | 1.22 | B | 1.33 | AB | 1.28 | AB | 1.31 | AB | 1.31 | AB | 1.36 | A | 0.13 | ns | 0.002 | ns | t |
| C18:1/C18:0 | 393 | 3.55 | B | 3.91 | AB | 3.43 | B | 3.89 | AB | 3.70 | AB | 3.87 | AB | 3.86 | AB | 4.10 | A | 0.58 | ns | 0.006 | t | ns |

BW= Birth weight, Nutri= Maternal Nutrition. LBW= Low birth-weight, NBW= Normal birth-weight. SFA = sum of saturated fatty acids, MUFA = sum of monounsaturated fatty acids, PUFA = sum of polyunsaturated fatty acids, UI = unsaturation index = 1 × (% monoenoics) + 2 × (% dienoics) + 3 × (% trienoics) + 4 × (% tetraenoics) + 5 × (% pentaenoics) + 6 × (% hexaenoics). RMSE = root-mean-square error. Ns= not significant, t= 0.1>*P*>0.05. Different letters in a line indicate significant differences (*P*<0.05).